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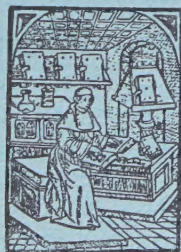
HYDROBIOLOGIA

ACTA HYDROBIOLOGICA HYDROGRAPHICA ET
PROTISTOLOGICA

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Letterstedtia insignis Areschoug

by

M. A. POCKOCK

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ARESCHOUG's beautiful species, *Letterstedtia insignis*, at its best the most striking and handsomest of all Green seaweeds, with its varied form and different shades of green, is at the same time probably the least well known.

ARESCHOUG (1850) based his description on material collected „near Port Natal” where he describes it as rare. His description forms the basis for the accounts given by J. AGARDH (1882) who also gives

the locality as „ex Port Natal” and DE TONI (1889) who states „Hab. in sinu natalensis Africae, rarius.” Thus, since ARESCHOUG’S material included holdfasts, it seems probable that the alga was actually growing in what is now Durban Bay.

BARTON (1893) in the first part of her list merely mentions it, stating „Port Natal fide Areschoug” but in the second part (1896) she gives three additional records from two other localities¹⁾: Port Elizabeth (WEBER VAN BOSSE), Port Alfred (SLAVIN) and the Kowie (BECKER). So far as the writer knows, *Letterstedtia* has not again been recorded from Durban Bay, nor any other part of the Natal coast, but it may well have been overlooked.

The alga is fairly well known in the form of herbarium specimens largely owing to TYSON and BECKER’S collections from the Kowie. But as a result of its habitat, its collection, attached, is difficult; probably therefore the majority of existing herbarium specimens (with the original material perhaps excepted) are from the drift. Hitherto little has been known of its habit, mode of life, development and reproduction. Of the latter in particular nothing whatsoever was known except for ARESCHOUG’S statement (l.c. p. 2) in his Latin diagnosis of the genus: „Schizogonidia in cellula foliorum formata.” This gives no indication of the significance of the varied thallus-forms in the plant, except that obviously the „leaves” are regarded as the only part concerned in reproduction.

Recently the validity of ARESCHOUG’S genus has been queried, PAPENFUSS (1954) having argued that it is not sufficiently distinct from *Ulva* to be maintained as a separate genus but should be sunk in *Ulva*. It has therefore become more than ever important that *Letterstedtia insignis*, the type species of the genus, about which so little apart from its external form, has hitherto been known, should be investigated, and that in particular its reproduction should be studied. Consequently, when, early in April 1958, magnificent growths of the alga were found to be easily accessible at low water of spring tides in Salt Vlei Bay near the Kowie (only 40 miles from Grahams-town by a good road), it was felt that this was an opportunity that should not be missed. Accordingly, since that date periodic visits, once or twice a month usually at spring tides, have been paid to Salt Vlei and to Waterloo Bay to study the alga actually growing and to collect material for more detailed study in the laboratory.

¹⁾ „Port Alfred” and „The Kowie” indicate the same locality. Port Alfred is the town-ship on the banks of the Kowie River near its mouth, „The Kowie” refers to the whole neighbourhood and has come to mean more specifically the adjacent coast. The chief collecting grounds are in that part of it lying between the mouth of the river and Kowie Point some five miles to the west.

OCCURRENCE

In the Eastern Cape Province on the coasts between Port Elizabeth and East London *Letterstedtia* is at times very common in the drift, conspicuous by its bright green colour and greater resistance to drying than *Ulva*. So fresh-looking and numerous are the specimens that it is obvious that the alga must be growing nearby and in abundance. Two such localities have been known to the writer for many years, Waterloo Bay just to the east of the mouth of the Great Fish River, where in April 1942 for the first time large plants were found actually growing, and Salt Vlei Bay some four or five miles west of the Kowie River mouth, where smaller bushy attached plants were first observed in February of the same year. It has also been found attached at Kwaai Hoek near Richmond (Feb. 1944), at Kleinemonde (Aug. 1943, Feb. 1947), in Jan. 1959 at Kenton-on-Sea (S. Greathead) and at Impekweni and several other places as far east as the mouth of the Keiskama River (B. Rennie). Eastwards towards Natal it probably occurs at many places where it has not yet been noted; exposed plants are usually bushy and easily mistaken for a large *Ulva*. If the original collecting ground was in truth Durban Bay, it is doubtful whether it could still survive there in view of the extensive harbour works and general commercialization of the area. As yet, Port Elizabeth is the most westerly station from which it has been recorded (WEBER VAN BOSSE). DELF & MICHELL's (1921 p. 2) tentative record of *Letterstedtia* from Kalk Bay is almost certainly a case of mistaken identity.

TOPOGRAPHY

As the present account is mainly concerned with Waterloo and Salt Vlei Bays a brief account of these two localities should be helpful.

Both are wide shallow bays with sandy beaches backed by sand dunes, a single stretch of sand at Salt Vlei, several interrupted by flattish outcrops of rock at the much more extensive Waterloo Bay.

Salt Vlei Bay gets its name from a pool formed by a small stream at its eastern end; this lies between sand dunes and is normally „blind”, cut off from the sea by a bank of sand. To the east the beach ends at Salt Vlei Point where extensive rock formations begin, extending eastward to Sharks Bay, TYSON's classic collecting ground, while at Salt Vlei they end in a series of broken rocky ridges, more or less parallel, running out to sea, with sandy gullies and pools between, all submerged or nearly so at high tide. Westward the sandy beach extends for about three quarters of a mile, the sand of the beach

piled up against low dunes which stretch some way inland, fixed by Port Jackson willow (*Acacia cyclops*). At high water the sea reaches nearly to the foot of the dunes. For three quarters of the bay the floor, even at low water, is sandy but in the last, western quarter the rocky substratum comes to the surface forming a broken terrain of small boulders and ridges with flatter rock between, the latter partially sandcovered and forming a series of small pools; the whole complex is as a rule submerged or wave-washed except at low water of springs when it may be more or less exposed for a short time. If there is much sea running, even then it is not easily accessible and at low water of neaps it is completely submerged, only a few of the upper rocks appearing between waves. The rock in this region belongs to the Witteberg Series and consists of much folded and faulted hard shales and quartzites, resulting in rugged formations, extending beyond the bay in the outcrop which forms Kowie Point. Further out, at low tide, waves can sometimes be seen breaking on a small reef lying parallel with the shore, normally quite inaccessible. Between it and the shore are sandbanks with deeper channels between them, the nearest one lying adjacent to the beach.

At Waterloo Bay a rocky headland lies east of the mouth of the Fish River, the valley of which here runs along the junction of the Witteberg and Karoo Series; eastward of this headland a wide sandy bay ends with a series of rather flat rocky outcrops through a gap in which a small river, the Old Woman's River, makes its way to the sea on the few occasions when the mouth is open; at other times the extensive lagoon it forms is completely cut off from the sea, winding inland between high dunes. Beyond the Old Woman's river the rocks extend for some way and it is with this part of the bay that the present account is chiefly concerned. Beyond the rocks stretches an even more extensive sandy bay, the whole complex of sandy bays and rocky outcrops comprised in the name Waterloo Bay.

The rock formation here is very different from that at Salt Vlei. The hard shales and mudstones of the Karoo System are more or less horizontally bedded, are very dark in colour and tend to weather into smooth platforms with large flat-floored pools with nearly vertical sides, not very deep. At intervals the rock is interrupted by sandy gullies and small bays. Here the dunes behind the beach are very much higher than at Salt Vlei, partly fixed with a typical dune flora behind which is coastal bush, partly still unfixed.

In both cases, the description refers to the normal condition of the foreshore but after stormy weather, and particularly after heavy rains, its whole aspect may be altered, only to return to the normal condition after a number of spring tides. Formerly Waterloo Bay was difficult of access from Grahamstown, reached by 35 miles of

good road followed by another thirty or forty miles of farm road; recently the building of a bridge at the Fish River mouth has nearly halved the distance, but even so the last few miles are practically impassable in wet weather.

In April 1942 *Letterstedtia* was spectacular at Waterloo Bay, growing in quantity in sandy gullies between parallel lines of small reefs running at right angles to the shore. The very long plants were completely submerged even at low tide, the long strap-shaped upper fronds waving to and fro with the ebb and flow of wavelets washing up the gullies. On the next visit, after heavy rains, no sign of *Letterstedtia* could be found. In January 1958 however, people walking along the beach reported it as very abundant and in the period here under review it has continued plentiful although never so large as in 1942.

FIELD OBSERVATIONS 1958

The alga is essentially a species of the sub-littoral zone and its fringe (STEPHENSON 1939 p. 502), reaching its optimum development in shallow bays where it is completely submerged even at low water of springs. In shallower water, often partially exposed at low tide, it is smaller and rather different in form. It may get up into intertidal pools but is then bushy and stunted, rather resembling a crisp lettuce in appearance (Pl. I, A) until short straps, similar to but shorter than those of the deep water form begin to develop from the upper margins of the fronds. Further, it seems to be markedly seasonal, the long „straps” common in drift material during the summer and autumn being completely absent in the later winter or early spring months. The persistent form, like that in pools, seems to be always bushy, the frilled straps only beginning to develop in summer. A complete contrast to the bushy plant is the typical deep water form (Pl. I, K), slender with characteristic long strap-shaped outgrowths from the upper part of the fronds, the whole reaching two or three feet in length (no plants reaching the length of „four or perhaps more feet” recorded by ARESCHOUG have yet been found).

In pools and shallow water the straps always remain comparatively short, often wide, while in deep water they may be a couple of feet in length and are often very narrow, sometimes as little as 3 mm. wide including the frills, which are then usually very closely plicate. Careful study of attached plants has revealed many intermediate forms and has added considerably to the knowledge of the external morphology.

The first visit of the present series to Salt Vlei, on the 5th. April, one day after full moon, there was a calm sea, no wind and a very

low tide. As the far end of the bay was approached it appeared as a sheet of green which on examination proved to be an extensive growth of *Letterstedtia*, almost unmixed with any other large algae, covering the exposed tops of small boulders and ridges and filling the small pools between them, while plants on the sides of the rocks hung down into the water. Plants on the tops of projecting rocks were bushy (Pl. I, B, D) and many were exposed for half an hour or more without showing any bleaching; those growing on the side of the rocks were also bushy at the base but most of them bore short straps hanging down into the pools, while in the pools themselves the straps were generally longer (Pl. I, C-F). Several small stones with *Letterstedtia* plants growing on them were extracted and taken back for more careful examination. Usually the surface of the rock was partly covered with encrusting corallines to which in most cases the algae were actually attached, not directly to the rock surface. Between the larger *Letterstedtia* plants were many young sporelings and somewhat older plants at all stages of development as well as several other algae, smaller and less conspicuous than *Letterstedtia*. These included a turflike species of *Gelidium*, *Falkenbergiella caespitosa*, a species of *Jania* and occasional small plants of *Gigartina pistillata*. Towards the seaward edge of the zone two species of *Codium* (*C. Duthieae* SILVA and a few plants of *C. extricatum* SILVA) and larger plants of *Gigartina pistillata* appeared among the *Letterstedtia* plants, entirely replacing them further out, but over most of the area *Letterstedtia* was dominant, without any of the larger algae admixed with it.

On examination, most of the *Letterstedtia* plants were found to be fertile; in the majority of cases liberation had already taken place and as the plants were handled the water in which they were being examined was filled with evacuated sectors and strips.

On the following three visits the growth of *Letterstedtia* continued the same, collecting varying with weather conditions. On the 20th. April constant waves made collecting difficult but there were many large plants with very long straps. At Salt Vlei Point a couple of young bushy plants, high up on an exposed ridge of rock, were collected, kept by themselves in a polythene bag; placed in fresh sea water the following morning they were found to be fertile; fully formed swarmers were seen moving within the parent cells and liberation from several fertile areas took place freely. The next visit, 4th. May the day of full moon, the tide was the best yet experienced and the sea so calm that large plants in the deeper water at the eastern end of the rocks were easily collected: still further east, as waves curled over to break on the beach, dark colour in the channel showed that the algal growth, mainly *Letterstedtia*, extended some way beyond low tide level.

A fortnight later, 21st. May, three days after new moon, there was a fairly heavy sea so that the tide was not very low and except at the far end of the beach near the rocks *Letterstedtia* could only be collected with difficulty between waves and even then the larger plants were inaccessible. Collecting was only possible for less than twenty minutes.

The 2nd. June, however, gave a totally different picture. After a week of stormy weather with exceptionally heavy rains the foreshore had altered to a surprising degree. Salt Vlei River was open to the sea having cut through the bank to the shore, then turning west parallel to the beach had formed a deep channel beyond which an extensive sandbank had formed, exposed at low water for most of its length; towards the middle of the bay this bank was breached by the stream but further west it was higher extending right out to the reef mentioned above so that it was possible to walk dryshod over the sand to the reef although normally the letter is almost completely submerged even at the lowest tide. In the channel were deep pools, unreached by the waves at low tide as was the complex of rocks and pools forming the main part of the *Letterstedtia* area. At the edge of the beach and at the edge of the sandbank beyond the channel many plants of *Letterstedtia* were partially buried in sand, the upper parts lying exposed on the surface of the sand; others no doubt were completely buried and hidden from sight. Beyond these partially exposed *Letterstedtia* plants were the upper parts of many plants of *Codium Duthieae* similarly half buried, several of them with *Letterstedtia* sporelings growing on them. Still further out, near the reef, both *Letterstedtia* and *Codium* were replaced by tufts of *Carpophyllum scalare*, buried to the last 6 to 12 inches, dotted about on the sandbank. Since in Salt Vlei Bay this alga reaches a length of 4 or 5 feet or even more, such buried plants provide an indication of the depth of the sand where they were growing.

The *Letterstedtia* plants both in the channel pools and in the main area were completely cut off from all wave action during the low tide period and attached evacuated fertile areas, rare here as a rule, were common on many of the plants, showing as colourless borders and sectors on the leaves and young fronds and as a narrow band along the margins of the frills of straps. It was also possible to collect large plants complete with holdfasts even from the deeper pools. Sporelings, some of them already large and becoming bushy, were more abundant than hitherto on the straps as well as on other algae (Pl. I, L).

The following two visits, 19th. June two days after new moon, and 1st. July, full moon, the foreshore was gradually returning to its normal condition but the inshore channel persisted, particularly

the large pool near its western end; collecting however was only possible near its edge.

At neap tide on the 10th. July the whole of the *Letterstedtia* area was submersed even at dead low water and it was only possible to snatch a few fronds from the uppermost rocks between waves.

On the 17th. August, two days after new moon, hardly any of the „*Letterstedtia* field” was visible, though it could not be determined whether this was due to silting up with sand or to the actual disappearance of the plants since the sea was running too strongly for closer investigation. A few rocks, exposed between waves, had bushy plants on them and in the channel pools some large plants with long straps could be seen but too deep to reach except momentarily at the edge. Most of the algal growth in the pool, however, now consisted of very large plants of *Codium Duthieae*. A few fronds of *Letterstedtia* were snatched between waves from plants at the edge of the pool; the bases were buried deep in sand and the straps were nearly destitute of leaflets, the frills reduced and the central region more or less covered with epiphytic growths. Ropes of twisted and intertwined straps showed the effect of the heavy seas while the absence of leaflets and the growth of encrusting Red and Brown algae, either on or in the outer walls of the central region of the straps, witnessed to their age. Only one fertile frond was found.

On the 28th. October, the day after full moon, the wind was strong and the sea heavy so that even at dead low water the tide was scarcely lower than at neap tide, except momentarily between waves. Very little *Letterstedtia* remained – no plants with straps were seen, only a few bushy plants and clusters of sporelings on the rocks and a few sporelings on *Codium Duthieae*. No fertile areas were seen on the bushy plants but several tiny sporelings on *Codium*, some only 4 mm. high showed eroded edges where fertile areas had been sloughed off, while in some cases, e.g. one sporeling about 6 mm. tall by 5 mm. wide besides several small fertile areas evacuated areas were still attached or beginning to slough off (fig. 4, F, G). Very large bushy plants of *Codium Duthieae*, scattered at intervals between the rocks, in the channels and even well up on the sandy beach, often buried deeply in the sand with only the tops of the fronds showing, now dominated the whole areas formerly covered with *Letterstedtia*.

During this period four visits were paid to Waterloo Bay. On the first, the 18th. May¹⁾, the wrong road was taken at first so that the low tide was unfortunately missed by 10 minutes and as there was a fairly strong sea running the tide was already flowing. The rocks near the Old Woman's River had a few bushy plants of *Letterstedtia*

¹⁾ For results of later visits, see page 62

still get-at-able. Further east it became more common; a gully in the rocks had large plants growing in it and towards the eastern end of the rocks it was abundant in the large flat pools. All the plants were bushy with comparatively short straps but the tide was already too high to reach the larger plants and no plants were seen in the open water. Practically all the plants examined were fertile, including several attached to small stones thrown up on the beach or in high-tide pools. In these the holdfasts were particularly well developed, broadly conical, coated and impregnated with fine dark silt from the Fish River.

On the second visit, the 2nd. July, the day after full moon, it was bright and sunny with a calm sea and conditions were altogether much more favorable. Most of the time was spent on the eastern rocks where there are many of the pools mentioned above – flat-bottomed, with nearly vertical sides a foot or more high. Round the edges and here and there on the rocky floor of these pools bushy plants with rather short broad straps were abundant, while further out, washed by waves even at low water, the plants were still bushy larger and with much longer straps (Pl. I, G). Nowhere, however, were seen such large plants as those found at Salt Vlei, and all the plants seen had bushy bases (Pl. I fig. C, G). In the deeper water the limit of the *Letterstedtia* zone was marked by boulders, just submerged, some covered with a dense growth of *Halimeda cuneata*, others with *Caulerpa filiformis*.

Low water was at 10.30 a.m. and soon after 11 o'clock, before the inorning tide had reached the pools, the water in many of them, particularly those just below the rather steep sandy beach, began to show a deep yellow-green colour where *Letterstedtia* was growing. Evidently liberation of swimmers was proceeding in enormous quantities. Presumably cold water percolating down through the sand was reaching the water of the pools, warmed by the sun, and was precipitating liberation. A quantity of the green water was collected in glass jars and polythene bags and taken back to Grahamstown. Examined microscopically the water was found to be full of still actively motile swimmers, mostly quadriflagellate, a few still biflagellate. Where eyespots could be detected two could be distinguished in the quadriflagellate swimmers, indicating their zygotic nature. Many were still motile the following morning, but though many settled down and rounded off on slides and cover-slips, comparatively few germinated. Probably the two hours drive in a warm car had lowered their viability.

A visit a week later at neap tide on the 9th. July was illuminating. As at Salt Vlei, at low water of neap tide all the *Letterstedtia* plants were completely submerged. As the waves receded it was only possi-

ble to reach a plant here and there at the uppermost limit of its growth.

On the 13th. September the foreshore here, as at Salt Vlei, had altered considerably; many of the pools were nearly filled with sand and it was difficult to identify those examined on previous visits. *Let-terstedtia* was less abundant but there were many young bushy plants as well as older ones of some size, with many straps, both broad and narrow but not very long. Again almost every plant examined was found to be fertile, with evacuated zones still *in situ*; the frills mostly showed a narrow colourless edge while many of the leaves and young fronds had colourless margins and sectors where liberation had taken place. The topography at Waterloo Bay results in greater protection from wave action than at Salt Vlei since the extensive rocks tend to moderate the effect of the waves until the tide is well advanced. Consequently the sloughing off of evacuated areas is delayed and they were usually fairly abundant here, but on this last visit they seemed to be even more common and more extensive than usual.

EXTERNAL MORPHOLOGY

Not one of the existing descriptions gives an adequate picture of the external form of the plant since all of them deal exclusively with the adult plant bearing long straps, usually broken off above the holdfast. ARESCHOUG (l.c. p. 2) it is true distinguishes between young and adult plants but his description is hardly helpful. Further, his description, followed by all subsequent writers, of the narrow strap-shaped portions as „stems” or „stipes” is misleading, since although they function as such in that they bear lateral leaf-like appendages, they are always flattened and are obviously merely narrow portions of the thallus. In the young sporeling as in *Ulva* a short terete stipe is present between the attachment organ and the expanded frond (fig. 4, A), but very early in development this stipe is completely merged with the holdfast from the top of which the flattened frond immediately arises (fig. 1, D). There is considerable variation in the form of the thallus – sometimes a plant consists of a single frond, sometimes of two or more emerging from the holdfast (Pl. II, A). If the plant is bushy, the frond is often widely expanded at the base becoming much lobed and dissected above, and finally, if the plant achieves the fully adult form, from the upper margins grow out the characteristic straps with their frilled edges (Pl. II G, J-L). Quite large bushy plants may consist of a single such expanded frond, but often there may be two or more, originating at a very early stage of development of the sporeling.

In other cases the fronds are narrow and strap-shaped at the base,

becoming foliose above and bearing long straps apically; again such a plant may consist of one such frond or of several arising from a single holdfast. Apparently such plants, although fully adult, are yet comparatively newly formed, whereas bushy plants may be several months old before they develop straps. Bushy plants are characteristic of tidal pools and shallower water and also appear to be the persistent form of the plant, found throughout the year; the narrow strap-shaped base is characteristic of deep water plants, particularly where the base is sand-covered (Pl. I, K, L). What evidence there is suggests that they are the quick growing summer form but further study is needed to elucidate fully the vagaries of plant form. At Salt Vlei such plants occurred mainly in the deep channel pools, bushy plants with short or long straps in the pools between the small boulders. At Waterloo Bay in 1942 the plants, fully submerged, were all of the long slender type; this year no plants were found there in more open water and all the plants in pools and even deeper channels were bushy even when the straps were comparatively long (Pl. I, G).

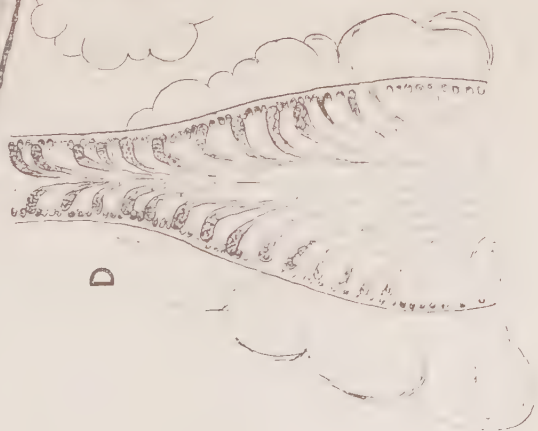
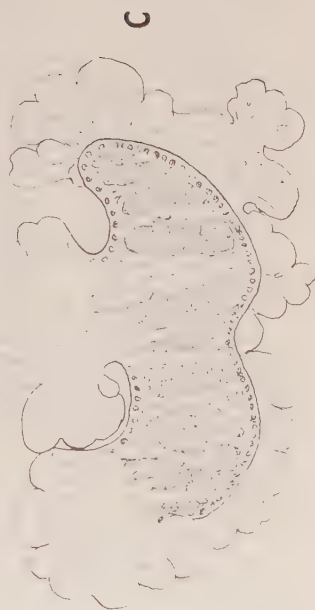
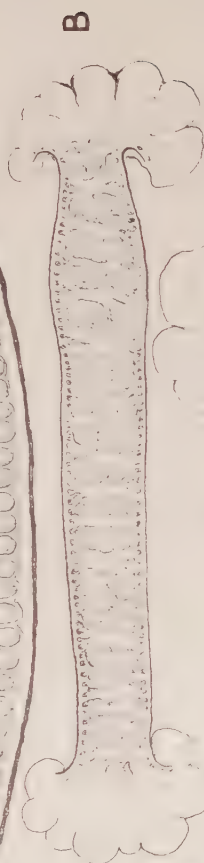
When first formed, for example in sporelings, the whole thallus is thin and much the same throughout. As it broadens the central part gets thicker and a paler green in colour, the margins and apices remaining thin and bright green. In the straps, even in very narrow ones, the central zone is fairly thick and light green, except where a midrib which appears a darker green is visible. The frills, especially their margins, always remain thin and bright green. Straps, even quite narrow ones, are strong and can withstand a considerable pulling strain. As a strap forms the meristematic marginal region elongates much more rapidly than the centre and is consequently thrown into folds, thus forming the characteristic frill which, contrary to most descriptions, is not toothed but plicate, the folding of the continuous margin giving the appearance of a toothed edge. Straps branch repeatedly by a kind of pseudo-dichotomy and bear two rows of lateral appendages arising at somewhat irregular intervals; these are leaf-like, thin in texture and bright green in colour and are usually but not invariably borne on short petiolar straps, similar to but narrower than the main straps (Pl. I, K, L). Here again, although the petiolar strap functions as a stalk to the leaflet, it is still a flattened thalloid structure.

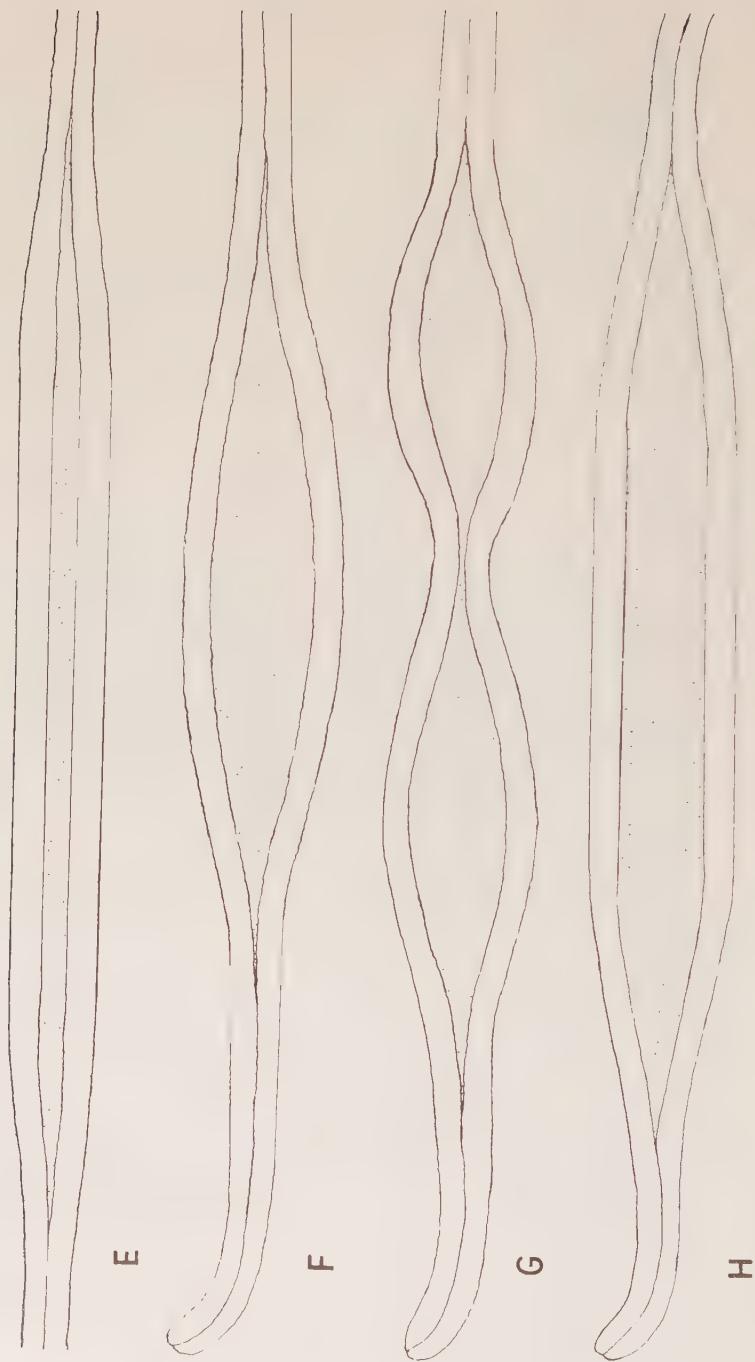
In quiet water or sheltered positions such as the Waterloo Bay pools the straps are often broad and may not show a distinct midrib. Sectioning however shows that the central region of the strap is nevertheless thickened (fig. 1, H). In open water, such as the channel pools at Salt Vlei the straps tend to be longer, much narrower with better developed frills, and usually have a pronounced midrib

Figure 1.

A Transverse section through midrib of strap, showing thickening. Thick-walled rhizoids filling interstices of trabecular system. B, C Transverse sections through lower part of frond: B. a short way above base, frond much thickened, edges protected by callus; C. just before entering holdfast, almost completely enveloped in callus, cell-layers distorted. D longitudinal section through base of sporeling showing attachment organ and stipe completely enveloped in callus, frond emerging from the top. E-H. diagrams of transverse sections of straps to show distribution of thickening: E. thin strap, thickening just beginning; F, strap with midrib; G, strap with two thickened ribs; H, narrow strap with thickening extending across central somatic zone.

A \times 170; B-D \times 38.5; E-H \times 86





(fig. I, A, F). In the later part of the season, as for instance on the 17th. August after rough weather the straps were often almost inextricably twisted and intertwined into a rope-like structure so that it was very difficult to disentangle them since not only is the surface not mucilaginous, but in addition the folds of the frills tend to lock the straps together.

Frequently the straps or their branches end in an expanded portion which divides to form two leaflets, thus originating a dichotomy. Yet a further complication is the ability of the lower part of the frond to give rise to leafy outgrowths from margins and spines of lobes, or even from the base in the holdfast, these again being thin in texture and bright green in colour (Pl. I, E, F). Often there is a great development of leaflets from the margins and still more so from the apices of the lobes of the thallus.

An examination of the basal part of the frond, particularly when it is broad, often reveals a system of lines slightly darker in colour than the rest of the frond and anastomosing downwards (Pl. I, G). These lines are comparable to but less conspicuous than the midribs of straps and eventually merge into a continuous zone of thickening across the base of the frond. In the straps the midribs may be intermittent or may extend throughout the greater part of the strap; at a fork the midribs of the two branches converge below it. The nature of these lines of thickening is more fully discussed in the section on anatomy, but externally they are a very characteristic feature.

Another curious feature is the formation of cartilaginous warts of a hyaline substance. These reach their greatest development in the holdfast but may also occur on the upper parts of plants, particularly the straps.

HOLDFAST OR ATTACHMENT ORGAN

Described by ARESCHOUG as „callus radicalis magnus”, the attachment organ is peculiarly characteristic. At its greatest development it is a somewhat flattened conical structure closely adherent to the substratum by a more or less circular base up to 2cm. in diameter, in height rather less. As it is coated and impregnated with arenaceous or calcareous particles it is very difficult to section when fully developed but in its younger stages it is more easily dealt with. The greater part consists of the hyaline substance characteristic of warts in other parts of the plant and it forms a highly efficient organ of attachment — plants thrown up on the beach are usually torn away above the holdfast, or if growing on small stones, the stone plus the attached

plant may be thrown up bodily. The mode of formation and internal structure are considered in greater detail in the section on anatomy and development of the sporeling.

CONSTITUTION OF A PLANT

If a bushy plant is examined carefully it is often found that the whole plant has developed from a single widely expanded frond, thrown into folds as it grows and the upper part much cut up, the margins delicate and meristematic and often bearing leaf-like or frond shaped outgrowths, while from the upper margins of the lobes straps eventually arise (Pl. I, B, C). In other cases there is more than one frond arising from the holdfast, or the original frond branches very early in its development (Pl. I, C, F, G). Examination of sporelings shows that from the beginning there may be a single little frond, or more than one, branching from the base of the primary frond taking place at a very early stage. A further complication is the ability of the margin of the frond, buried in the holdfast with the two cell layers widely separated, to give rise to lateral outgrowths which may develop into supplementary fronds.

Finally there is some evidence from spore cultures to show that a single „plant” may in fact be composite, formed from a cluster of swarmers germinating in such close juxtaposition that they fuse to form a single plant. It is possible that the summer form described above is sometimes of this nature or else the plant is one in which several branches are formed at the base, growing rapidly so that the lower part remains thin forming a narrow strap-shaped structure almost unthickened — the „stem” of the earlier descriptions. It was probably with such plants that ARESCHOUGH was dealing since he makes no mention of bushy plants (Pl. I, K, L).

ANATOMY

a. Primary Structure.

Transverse sections of leaflets, young straps and upper parts of the expanded fronds, like the young sporelings, show an *Ulva*-like construction in that they consist of two layers of cells elongated at right angles to the surface with a thick common membrane on the outside, and this is the basic structure throughout the plant. But it differs from *Ulva* in that the walls of individual cells always remain distinctly differentiated and furthermore that the two rows of cells are in close juxtaposition, the inner walls being in contact and even partially fused with one another; here and there intercellular spaces are

evident but there is no amorphous hyaline zone between the two cell layers. Further, except in the youngest parts the walls are noticeably thick.

All the bright green portions of the plant - young leaflets and fronds, frilled edges of the straps, margins of the fronds - are young and retain the power of division and growth, that is, they are meristematic. The cell walls are thin; each cell contains a single chloroplast which lines the outer two thirds of the cell but usually not filling it, and encloses two or more pyrenoids. In surface view actively dividing cells appear half green, half colourless, the colourless parts of two cells lying adjacent to one another, the wall between them having been formed when the parent cell divided and usually being thinner than the other walls (fig. 2, A. C). Division follows no definite rule, except that it is always in a plane at right angles to the surface so that the thallus always remains two layered. Throughout, this primary structure persists in all young and hence meristematic parts, whether they be near the base or in the distal parts of the plant.

b. Differentiation of permanent tissue.

The change in cell structure as young tissue ceases to be meristematic and becomes somatic or permanent is best seen in transverse sections of the straps (Pl. II, B). The frill is meristematic, the outer cells constantly adding to the meristematic region while towards the inside the cells become somatic, adding to the central zone of the strap. As this happens, the wall of the cell thickens, the inner wall becomes more closely united with those of the opposite cell layer, the cell lengthens and the single chloroplast breaks up into a number of granular plastids, the colour consequently changing from the homogeneous bright green of the single chloroplast to a paler less clear somewhat yellowish green (fig. 2, B). In the broader fronds the margins and tips remain meristematic while the central part becomes somatic.

Somatic cells have apparently lost the power of division but not of growth, and elongation of the cell and changes in the wall continue. Further, some among them are distinguished by richer content with deep green granular chloroplasts and by their position, slightly sunk below the level of the adjacent cells; it is these cells which give rise to the rhizoidal processes (Fig. 1, D).

c. Growth in thickness.

Somatic tissue may continue in this state, for example, in the central zone of the ligulate basal parts of plants growing in sand and sometimes in young straps, but more usually growth in thickness soon begins. Again it is easiest to study the transition from newly

Plate I

Letterstedtia insignis. External Morphology.

A, young bushy plant. B, plant attached to small rock, base bushy, upper part with developing straps. C, young bushy plant with many straps developing from upper part of thallus; base expanded, divided into several parts. D, small rock with attached plant removed from pool for photographing, showing long straps growing from bushy base; in the water these hang down into the pool. E, young plant with many delicate fronds in lower part, straps with leaflets above; typical of rapid growth. F, G, well developed bushy plants with many straps, typical of fairly deep pools; in F „loops” in the straps show clearly, many young fronds below, leaflets above, expanded base of frond divided into several branches. In G a single ribbon shaped base arises from a large holdfast, giving rise to lateral branches; in the largest several ribs of thickening. H, part of a large bushy plant from drift with repeatedly divided frond; midribs particularly clear. J-L, fully adult plants, all with undivided base in which the thallus is stalk-like in appearance, though actually flat. In J, from deep pool, the holdfast is large and shows clearly. In K & L from the deep channel, base was sandcovered and is slender with small holdfast, and several fronds, dividing above into two or more branches. In L, older than K, there several sporelings on the straps one group at the apex of the longest strap already fertile, straps almost denuded of leaflets which are still present in K.

C, G, H from Waterloo Bay, the rest from Salt Vlei Bay.

B, D about 1/10 life size, the rest all about 1/6 life size



formed somatic cells in unthickened tissue to well advanced thickening in young straps.

Since in the Ulvaceae there is no tangential division of the cells even in large plants, there is no growth in thickness in the ordinary sense and strengthening must be attained in some other way. In *Ulva* and *Monostroma* the only method is by the putting out of rhizoidal processes at their inner end by some of the cells. In *Monostroma* this is confined to the stipe and the central part of the thallus immediately above the stipe. In *Ulva* it results in a pushing apart of the two cell layers and the interpolation between them of a mass of rhizoids resulting in an appreciable thickening of the thallus both in the stipe and in the central part of the frond above the stipe. In *Letterstedtia* on the other hand there is a two-fold process of thickening which may occur in any part of the plant whether basal or distal: 1) As in *Ulva* some of the cells put out rhizoidal processes which grow downwards between the two layers of cells and 2) At the same time the cells elongate, wall-substance is laid down in the inner walls and where opposite walls are already united a cross strut develops, like the rhizoids pushing the two cell layers apart, but at the same time connecting them. The resultant struts or trabeculae may become complex structures derived from more than two cells, forking several times and developing lateral outgrowths, the interstices between trabeculae being filled with closely packed rhizoids. In transverse section the rhizoids are more or less circular in outline but flattened by mutual pressure, varying in size according to the distance from the parent cell, with very thick walls and small generally colourless lumen; the chloroplasts of the parent cells extend into the rhizoidal outgrowth for a short distance only, hence only the larger rhizoids show pigment. Occasionally the lumen of the parent cell from which a trabecula has originated is considerably elongated inwards but usually it remains the normal length, the inner end abutting on the much thickened wall. Further, as development proceeds the deposition of wall substance extends among the rhizoids so that they appear to be buried in the substance of the trabeculae, wall of rhizoid and trabecular substance more or less completely fused. (fig. 1, A; Pl. II, E, F).

In the straps growth in thickness may be concentrated in a single central midrib, tapering off on both sides, trabeculae progressively shorter and rhizoids fewer, until there are only very short cross-struts with here and there a solitary rhizoid, then only rather thickened fused inner walls until finally the two layers are in close contact, with inner walls partly fused and occasional angular intercellular spaces; finally such typical young somatic tissue passes into the meristem of the frill (fig. 1, F).

As cells in the opposite layers do not exactly correspond, the end wall of one cell may be in contact with the end walls of several cells of the opposite layer. Consequently several cells may contribute to the formation of each trabecula. The whole process of thickening may thus be studied in a single section (fig. 1, A). In other cases instead of a midrib, a strap may have two or more lines of thickening (fig. 1, G), while straps which show no ribs at all may on sectioning be found to have the whole of the inner region thickened; this seems to be often the case in the narrow petiolar straps (fig. 1, H). Finally in very thin straps the only thickening is due to a slight elongation of the fused inner walls unaccompanied by any rhizoid formation (fig. 1, E).

d. Nature of the Wall-substance.

The chemical constitution of the various parts of the walls and trabeculae has not yet been investigated. That it differs in different parts is well seen if transverse sections of fresh material are treated with an aqueous solution of iodine in potassium iodide. In thickened areas the result is spectacular. In the meristematic region the common wall shows a thin outer skin or „cuticle” which stains brown. Beneath this is a thin laminated yellow layer, while the walls of the cells themselves stain a greyish blue, strong towards the outside, shading gradually to paler blue in the innermost walls, fused here and there with the corresponding walls of the opposite layer. As the transition to somatic areas takes place, the yellow inner part of the common wall begins to thicken and the cells to elongate; their walls are still blue as in the younger zone, but the fused portions of the inner walls which are beginning to thicken and elongate are paler in colour.

In the central zone of thickened tissue forming the midrib the „cuticle” of the common wall is still thin and stained brown while the inner bright yellow layer is much thicker. The cell walls are deep grey blue on the outside, the colour extending into the side walls but becoming much lighter inwards until in the fused portions it changes from pale greenish blue adjacent to the cell lumen to bright yellow in the trabeculae, while the walls of the rhizoids are a pale pinkish mauve. The contents of the cells and of those rhizoids which still have any are a deep bluish black due to the starch content.

Transition zones between thickened and unthickened parts give similar coloration but as one passes outwards and the trabeculae shorten, the yellow colour gradually disappears until it eventually merges into the pale greenish-blue colour of the fused walls between the two cell layers.

In surface view the cell walls stain blue and are more or less thicken-

ed according to their position in the various parts, the thin wall between recently divided cells remaining unstained.

e. Distribution of the thickening.

It must be emphasized that unlike *Monostroma* and *Ulva*, where in the former thickening is confined to the stipe, and in the latter to stipe and central part of the thallus near the base, in *Letterstedtia* it may occur in any part of the plant, whether base of the frond or extreme tips of the straps. In the young sporeling, as in *Ulva*, rhizoidal thickening is found in the short stipe and base of the little frond but as development proceeds and the plant becomes complex morphologically, thickened tissue develops in all parts of the plant, the young meristematic regions alone excepted.

The midrib varies very much in distinctness; it is much more strongly developed in plants growing in open water – at Waterloo Bay where most of the plants examined were growing in pools partially sheltered from the full force of the waves midribs were much less noticeable than at Salt Vlei – but on sectioning apparently ribless straps thickening was found to be present, spread across the greater part of the somatic zone instead of being concentrated in the centre. Further, the midrib in any strap may be intermittent, very distinct for a space, then apparently disappearing where a more diffused zone of thickening occurs; in other straps the midrib is continuous. Below a fork the ribs of the two branches usually anastomose into a single wider rib. In wider straps there may be two or more ribs or the thickening may be spread across the somatic region, gradually diminishing towards the flanks (Pl. I, F, G, H).

In the basal fronds where the frond is wide, a number of faintly darker lines may be visible externally (Pl. I, G), anastomosing towards the base until a thickened zone extends across the frond from edge to edge. Where the frond narrows to enter the holdfast the whole of it may be thickened and often further strengthened by wart-like surface secretions extending up the margins or even entirely covering it. These warts are cartilaginous and similar in substance to the trabeculae giving a slightly less bright yellow with iodine (fig. 1, B & C).

As mentioned above, in the case of newly formed yet fully adult summer plants, particularly when growing in sand, the base of the frond may be narrow and strapshaped with little or no thickening, several such fronds arising from the common holdfast to constitute a single plant. In such cases the unthickened base just above the holdfast is a line of weakness and frequently such fronds occur in the drift broken off just above the attachment organ. But as a rule even in such thin straps there is some incipient strengthening, the fused

inner wall being slightly thickened and elongated although there are no associated rhizoids (fig. 1, E. Pl. 2, B), and later the thickening may develop further. The upper strap shaped appendages of the plant are surprisingly strong, even the narrowest strap possessing considerable tensile strength combined with extreme flexibility. In the case of bushy plants with an expanded basal frond the entire base is noticeably thickened and leathery to the touch.

f. Anatomy of the holdfast.

The internal structure of the organ of attachment is best studied in young sporelings epiphytic on straps since here, though well developed, it is less sand-encrusted and consequently more easily sectioned. To begin with, the plantlet shows an expanded frond, a short terete stipe and a basal attachment organ (fig. 4, F, G, fig. 5, A). In the base of the frond rhizoidal cells are clearly distinguishable in surface view and in section the rhizoidal outgrowths are seen to grow downwards into the stipe, pushing apart the cell layers and ending in a pad closely adherent to the strap. Often the sporeling develops on the frill and the attachment organ may actually wrap round the edge of the frill (Pl. II, A).

Early in the development of the sporeling the attachment organ becomes enveloped in a hyaline callus secreted by the basal cells. As development continues this is progressively more developed until it forms a wart-like structure surrounding the holdfast and extending upwards until it completely envelopes the little stipe. Meanwhile secretion of wall substance is also taking place internally between the primary cell layers, partly in the form of trabeculae, partly as somewhat amorphous deposits pushing the still green cells far out towards the surface, while the whole mass is traversed by greatly attenuated rhizoids (fig. 1, D). Sometimes the callus, both internal and external, has a cellular appearance as though formed of greatly blown up cells but this appearance is deceptive, caused by successive depositions of arcs of wall substance. The cells, although widely separated and pushed out towards the surface, the two layers much distorted, are still green and active and no doubt control the secretions of callus. Further, from them may arise lateral branches of the frond, at first rod-like, later expanding into flattened secondary fronds. In spore cultures rounded secretions of hyaline refractive substance were frequently formed on the surface of cells; although in this case it was certainly in part at least pathological, it is interesting as showing the mode of origin of the cartilaginous warts (fig. 3, U).

Quite young attachment organs may begin to get sand-encrusted, the sand particles adhering to the mucilaginous surface of the callus; as secretion of callus continues, the sand gets overlaid by new materi-

Plate II

Plate II Letterstedtia insignis. Anatomy.

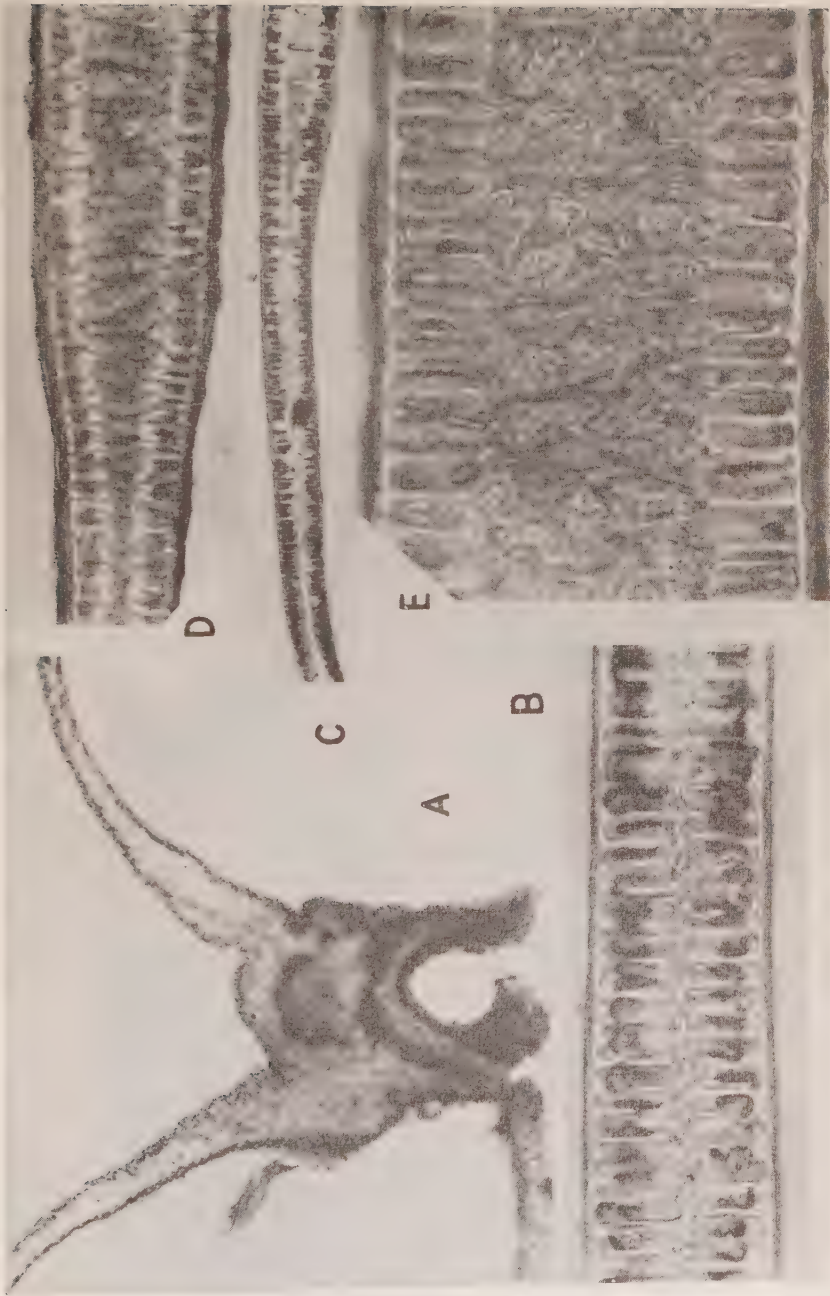
A, base of sporeling epiphytic on frill of strap; longitudinal section showing massive holdfast wrapping round frill, two fronds arising from it.

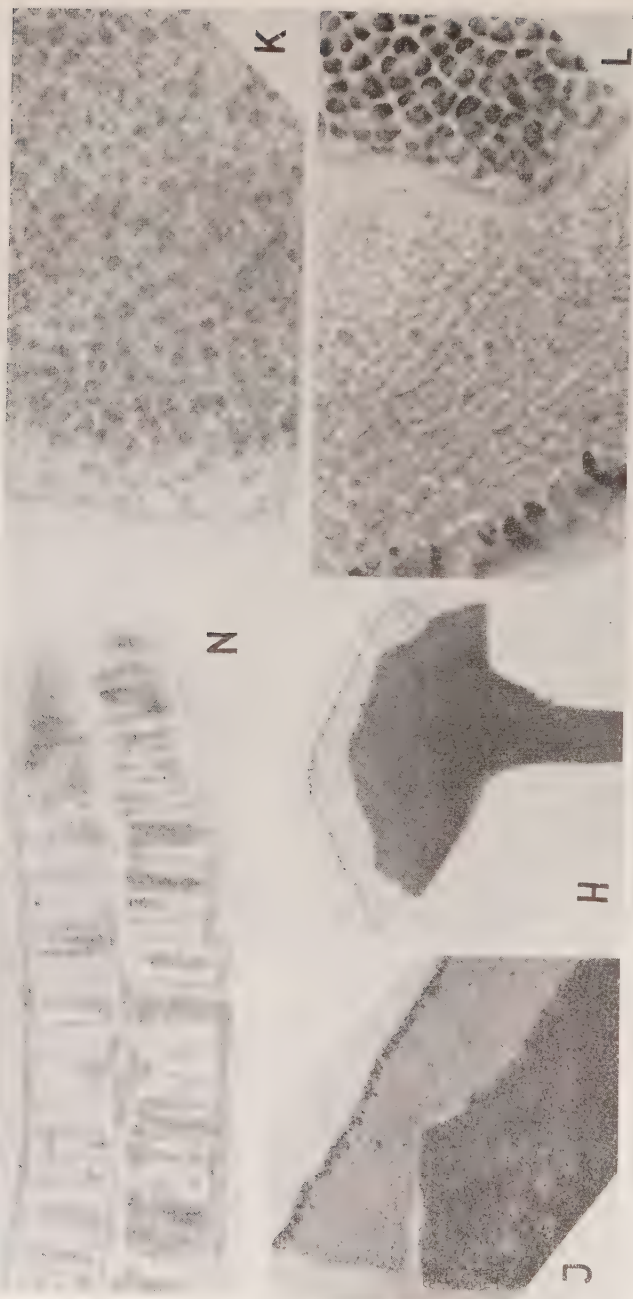
B-F, structure of the thallus, transverse sections: B, young strap showing inner walls in close contact, cells on left becoming somatic; C, D, sections through straps showing thickened midrib; E, F, detail of thickening, E, part of midrib showing trabeculae with interstices packed with thick walled rhizoids cut transversely, F part of same more highly magnified.

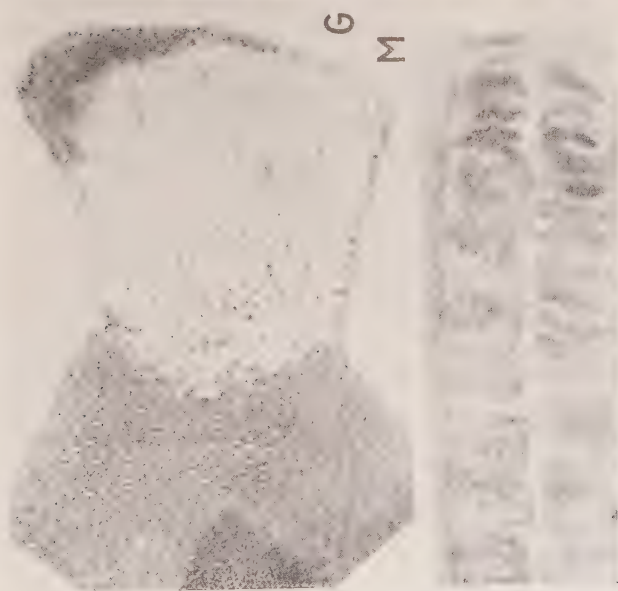
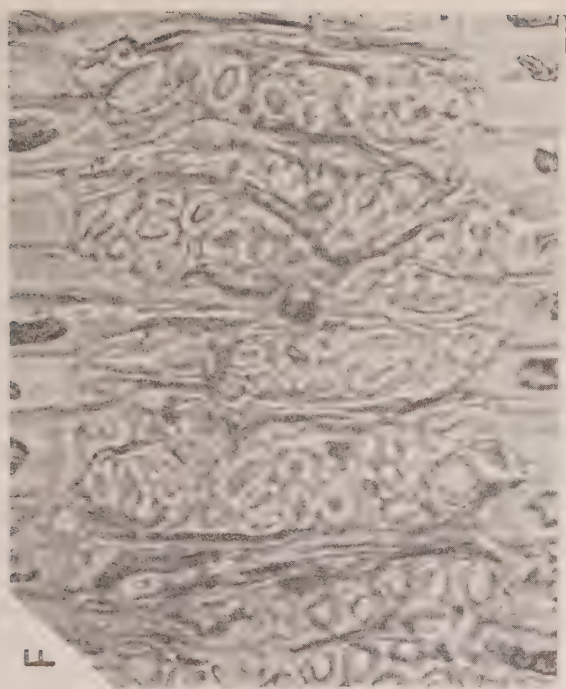
G-N, Reproduction, all from frills of straps: G, unusual limited fertile segment showing evacuated area with border cells on three sides, very wide in parts, some gametangia still with contents; on left two zones in different stages of development- in lighter area gametangia are developing, in small dark patch cells are still dividing actively; H, surface view of small portion of fertile strap showing fanning out of frill, evacuated fertile strip beginning to slough off, border distinct; J, edge more highly magnified to show beginning of separation of fertile zone from the frill, K, L surface view of parts of fertile frills; in K, border cells appear light, fertile cells in process of gamete formation, in some protoplasts dividing, in others gametes already formed and rounded off; in L, evacuated fertile strip beginning to separate, border cells dark, a few gametes still within parent cells, others rounded off on surface (top left), adjacent cells of frill recently divided preparatory to differentiation of gametes. M, N, transverse sections of edge of fertile frills showing gametangia, some already empty; border cells with darker contents on right, out of focus.

H \times 26 approx.; A, C \times 38; G, J \times 53; D \times 105; B, E, K, L \times 220;

F, M, N \times 440







al so that the whole structure becomes sand impregnated as well as encrusted. Eventually the holdfast may reach a considerable size which may vary from place to place, as does the nature of the sand-encrustment; at Salt Vlei Bay the holdfasts were coated with relatively large light-coloured particles of sand, while at Waterloo Bay the particles, derived from the silt brought down by the Fish River, were much finer and darker than those formed from the Witteberg quartzites.

From the summit of the holdfast the flattened fronds arise directly, the base usually protected by an extension of the callus, and the structure and mode of origin of this callus is best studied in a series of sections through such a base.

The thickening extends right across the base of the thallus as a broad band of a complex of trabeculae and rhizoids, while the edge of the frond is protected by callus; nearer the base this callus becomes increasingly extensive until where the frond enters the holdfast it is completely enclosed in hyaline callus-substance (fig. 1, B & C), usually less sand-encrusted than the holdfast; when the latter is cut it is obvious that the system of thickening and callus-formation is continuous with that of the frond, but in the holdfast the rhizoids are more attenuated and ramify in all directions while the trabeculae are less definite in form. The reaction with iodine is similar but the yellow colour in the holdfast is lighter and less bright than in the straps.

If the frond is a very broad one secondary attachment organs often form along the lower edge, beginning with the secretion of callus. The warts which are sometimes found on the upper parts of the alga are similar in structure and like the holdfast may become sand-encrusted, sometimes to such an extent that they have a stone-like appearance, but in their case the stony layer can usually be flaked off leaving the horny core which is usually brownish in colour. All such warts as yet sectioned have been found to contain the fine filamentous Brown alga already mentioned, ramifying through the substance of the wart and over its surface. It seems possible that the warts on the upper part of the frond may be of the nature of galls, formed in response to some external irritant, whether plant or animal; in other cases secretion of callus appears to begin in response to a contact stimulus, as for instance the secondary attachment organs on the edge of the frond and in spore cultures. The same Brown alga is sometimes found in the holdfast itself as well as on the walls of older straps where it appears to be partly endophytic in the outer wall, partly superficial.

REPRODUCTION

It has generally been assumed that the characteristic straps with their frilled edges and lateral leaflets were directly concerned in reproduction, but apart from ARESCHOUGH's statement that „Schizogonidia" were formed in the cells of the leaflets there is nothing in the literature to indicate the nature of the reproductive processes nor in what parts of the plant they actually occur. Search for reproductive bodies and attempts to induce reproduction in the laboratory had hitherto met with no success. Hence the finding in April 1958 of plants in a state of active reproduction was particularly gratifying. This has been followed by evidence of extensive reproduction at every subsequent visit at spring tides. Even on the most recent visit to Waterloo Bay on the 13th. September, most of the plants showed to a surprising extent recently evacuated areas still *in situ*.

Reproduction is not confined to the leaves as ARESCHOUGH's statement suggests; on the contrary, it may take place in any young meristematic part, no matter where it occurs in the plant. As already indicated, in the adult plant such regions include the frills and leaflets of the straps, margins of the older fronds and such young lateral appendages – leaflets, fronds, etc. – as may arise therefrom, often even near the base where young fronds are still thin, any part may become fertile even the central regions.

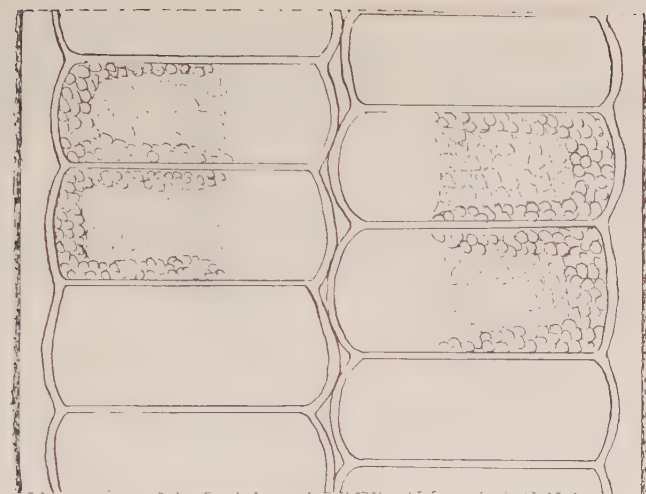
Quite young sporelings less than a centimetre tall may become fertile (fig. 4, A). The whole of the frond is still meristematic, the base alone excepted, so that fertile areas may be formed at any spot. Sometimes the whole distal region becomes fertile and after evacuation the sloughing off of the now colourless fertile area leaves the frond truncated or even bilobed. If fertile areas form medianly here, as in young lateral fronds, a row of holes may result, leading to a split in the frond, the initiation of laciniation (fig. 4, B). If the fertile areas are marginal, they leave the frond with eroded margins, all contributing to the evolution of a complex plant (fig. 3, F, G). With age, progressively more of the primary frond and later of its appendages becomes somatic and the extent of reproductive regions proportionately less.

Differentiation of a fertile area always takes place in a meristem in which the cells have recently divided. In surface view such recently divided cells are most characteristic in appearance, half-green, half colourless, the colourless parts adjacent to one another, separated by a thin wall which remains unstained with iodine (fig. 2, C). Each fertile area is sharply delimited. The cells of the outermost two or three rows do not divide; they retain their single chloroplast and the outer wall thickens somewhat (fig. 2, D). At the inner limit of the

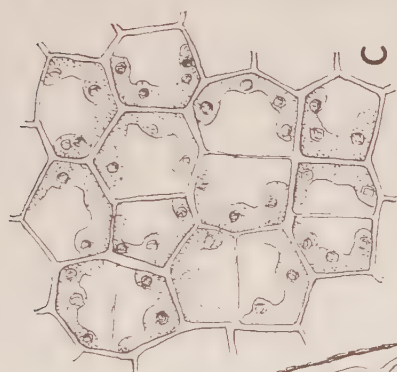
Figure 2

Anatomy and Reproduction: A, B, E transverse sections through strap, A edge with meristematic cells, two just divided; B, edge of central zone showing somatic cells; E, outer part of frill showing meristematic cells and gametangia, some already empty. C, D, F, M, surface views: C, meristem with recently divided cells; D, edge of fertile area showing two rows of border cells and gametangia; F, empty gametangia showing pore, with gametes and zygotes settled down on surface and rounded off; M, two gametangia with large cells, each formed by fusion of two immature gametes. G, gametes; H, conjugation nearly completed; J, planozygote; K, L, zygotes newly rounded off; N, zygospore, wall secreted but eyespots still conspicuous. O-Q, germination, elongation beginning, eyespots still conspicuous; in Q spore has enlarged considerably and contents have become granular prior to commencement of elongation.

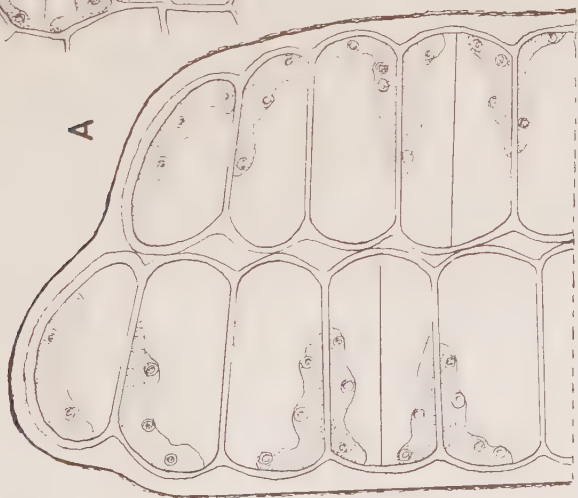
G-L, N-Q $\times 1700$; the rest all $\times 850$



B



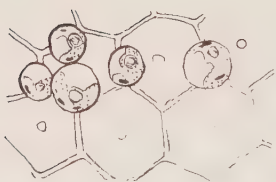
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A



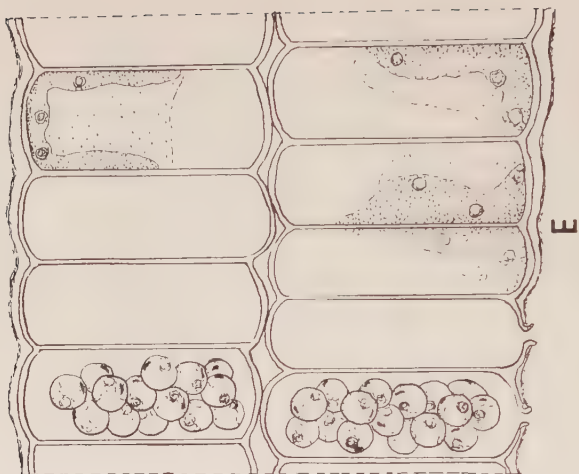
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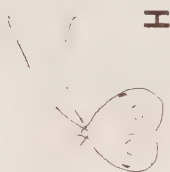
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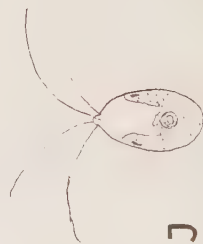
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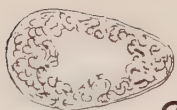
O



P



Q



area a similar change takes place in the cells of the next two or three rows to form what will become the new border after evacuation and sloughing off of the fertile area. Between these two borders all the cells are more or less simultaneously transformed into reproductive cells (Pl. II, G-H). In each the nucleus divides four or occasionally five times in succession, each nuclear division being followed by cleavage of the cytoplasm. Each part rounds off, shows a parietal chloroplast with a single pyrenoid and conspicuous eyespot. They are now ready for the final transformation into swimmers. At this stage, the frond is from 72 to 90 μ in thickness, with thin walled fertile cells 13—22 $\mu \times$ 26 — 39 μ , and each rounded immature swimmer 5—6 μ in diameter (fig. 2, D, E). Eventually each part puts out two flagella, becomes elongated, and as the time for liberation approaches, begins to move within the parent cell in which contraction of the protoplast during their formation has allowed room for movement.

Meanwhile in the centre of the outer wall a small slightly raised papilla has been formed in the apex of which a pore opens to allow the swimmers to escape. (fig. 2, E, F). Movement of the swimmers within the parent cell has been observed two or three times but their actual escape has not been seen. Since maturation and escape depend to some extent on external factors, it is not easy to find just the right stages.

When fertile plants were first found (5th. April) only a few swimmers were free in the water and conjugation was not observed. But many had settled down on the surface of fertile parts and rounded off. Some were small (5—6 μ) with a single eyespot, others larger (7—9 μ) with two eyespots (fig. 2, F, M). Pieces of such fertile leaflets were put in a culture dish in fresh filtered sea water and kept under observation. Many of the spores began to germinate almost immediately, the eyespots remaining conspicuous even after elongation had begun. Most of the germinating spores were small with one eyespot, others larger, with two. Unfortunately germination did not get beyond the initial stage of enlargement and most of the spores soon degenerated. Further, when material was examined two or three days after collection it was found that in many of the fertile cells there were fewer than sixteen daughter cells. But in such cases some were always larger than the normal size and contained two or more eyespots, indicating that after formation of the daughter cells and differentiation of their constituent parts, but before the emission of flagella, fusions had taken place within the parent cell. Obviously however, this cannot be regarded as normal conjugation within a gametangium but a pathological phenomenon due to abnormal conditions.

On the whole therefore, as yet the evidence as to the nature of the

swarmers was inconclusive; if they were gametes it seemed probable that they were facultative and capable of germination without conjugation. Since germination did not proceed beyond the initial stage, however, this has not been confirmed.

On the second visit (20th. April) two young bushy plants were collected from an exposed reef at Salt Vlei rocks, brought back in a polythene bag and the following morning put in fresh filtered sea water. Both were found to be fertile and when portions of the upper lobes of the fronds were mounted in sea water and examined, swarmers were seen moving vigorously within the parent cells ($17-20 \times 49-54 \mu$) and liberation was obtained, „clumping” appearing among the liberated swarmers almost immediately; small rapidly rotating groups of some five or six swarmers ($3.5-5 \mu \times 7-9 \mu$, flagella $9-10 \mu$) working busily, quickly formed; after a short time, members of a group scattered leaving the copulating pair (fig. 2, G, H). Actual union must take place very rapidly since very few pairs in the act of uniting could be seen, but soon quadriflagellate planozygotes were abundant – as evidenced by iodine fixed samples. Examination in a hanging drop, using the $1/12''$ Oil immersion lens showed two stigmas in each (fig. 2, J). Planozygotes were allowed to collect on slides where they settled down and rounded off. (fig. 2, K, L). The results of the germination experiments which were started are described later.

Finally at Waterloo Bay on the 2nd. July as described above, mass liberation was observed in the field. Here the trigger mechanism which started the process of liberation was not the sudden advent of fresh sea water on the incoming tide but the impact of cold water percolating through the sand of the beach on the sun-warmed water of the pools in which *Letterstedtia* was growing.

During the present series of observations, until late in the year, it was obvious that the *Letterstedtia* population was constantly being added to since young plants and sporelings of all ages have been abundant, whether on rocks or in the case of the latter epiphytic on *Letterstedtia* itself or on other algae. It seems obvious that such young plants and sporelings were all the product of the current year's activity, formed recently either from zygotes or from azygotic cells. As yet no evidence of quadriflagellate zoospores has been found. Sporelings of various ages became abundant as the year advanced, particularly those growing epiphytically either on the straps or on other algae, such as *Codium Duthieae*, *Gymnogongrus* sp., *Gigartina pistillata*, *Plocamium corallorhiza*, *Hypnea spicifera* and *Dictyopteris longifolia*. On the last named several large sporelings already bushy and fertile were found during the last visit to Waterloo Bay on the 14th. September.

Fate of the reproductive areas.

The comparative rarity of colourless rims or patches left by escaping swimmers was very noticeable at Salt Vlei, though even there it is subject to considerable variation. Typically all the cells in a fertile area, always excepting those of the border, mature and are evacuated simultaneously so that the whole area consists of empty and therefore colourless cells.

With the incoming tide wave action resulting in the whipping of the straps and other upper parts of the frond causes the evacuated area to strip away from the adjacent tissue, the innermost row of empty cells acting as a line of abscission, while the next few rows of cells are already prepared to form the new margin. (Pl. II, G-H). Successive reproductive areas often overlap and a new fertile area may be in active preparation just within the evacuated patch (Pl. II, C). In leaves and young fronds the sloughing off of successive arcs of emptied cells results in characteristically eroded margins. In the straps usually a continuous narrow marginal strip of the frill becomes fertile, finally sloughing off as a slender undulate ribbon sometimes several centimetres long; later less continuous strips become fertile and in turn liberate swimmers (Pl. II, H). The whole process, combined with the continual increase in length brought about by the increasing size of the somatic cells and in breadth through the activity of the inner cells of the marginal meristem, results in a progressive narrowing and smoothing out of the frill, the central somatic region meanwhile increasing in breadth.

The spring tide at the beginning of April seems to have coincided with a peak period of reproduction at Salt Vlei; as plants were being prepared for mounting narrow undulate ribbons were constantly being stripped from the frills and arcs or lunate pieces from the leaflets so that the water in which the plants had been lying was full of little ghost-like pieces of thallus, apparently quite colourless. Under the microscope however all showed a narrow green rim one or two cells wide on the outer margin, while the inner margin showed a ragged line where the cells had torn away. Although the border cells had not formed gametangia when the area first became fertile, when kept in culture in many cases they too divided to form swimmers. Here and there among the empty cells are often scattered solitary cells which have either not divided or have failed to liberate their swimmers (Pl. II, G). In surface view the pore shows clearly in the centre of the wall of the empty cell, sometimes as a somewhat ragged hole (fig. 2, F) while in section the appearance of the papilla with its terminal pore is similar to that figured and described for *Ulva* (fig. 2, E).

On the 20th. April fertile areas, although present, were much

smaller and less abundant than at the previous visit; on the 4th. May reproductive areas were again abundant but frequently immature and much smaller than on the first visit. Liberation from some of the material was sufficient to colour the water green, but the sloughed reproductive pieces were small and in many cases still partly green, liberation not having been complete. This last tide was even lower than that of the 5th. April and much of the *Letterstedtia* was at least partially exposed at low water.

The sloughing off of reproductive areas subsequent to the liberation of the swarmers takes place naturally as the result of wave action on the incoming tide; as the tide flows and the plants are more deeply submerged the effect of wave action gradually decreases, unless the sea is a very heavy one. The sloughing process is a most characteristic one and accounts for much of the irregularity of outline in leaflets and fronds as they age, the changes in the form of the frills and for the usual absence of extensive colourless zones to mark reproductive areas in the plants. The exact delimitation of such areas is also characteristic: there is never a progressive formation of fertile cells from the margin inwards such as is described for *Ulva*, each reproductive zone being clearly delimited and separated from any successive ones by the border cells which mark its inner limit.

The persistence of evacuated zones varies considerably. In Salt Vlei Bay where most plants are normally subject to wave action even at low water attached colourless areas were comparatively rare, but on the 2nd. June when the changes in the foreshore had resulted in the complete isolation at low water of the whole of the *Letterstedtia* grounds from the open sea, most of the plants showed colourless edges and patches. At Waterloo Bay again where the full force of the incoming tide is somewhat broken by the rock formation, evacuated zones were much more persistent and even as late as the 13th. September nearly every plant showed numerous extensive colourless edges to the frills and arcs and marginal zones on the leaflets and young fronds, easily seen in hand specimens.

When fertile areas develop medianly in young fronds or frond lobes, the evacuated areas may leave holes as they are shed (fig. 4, B) and it is common to find in such cases that the margin of the resultant hole or split if one occurs may develop a frill similar to those on the straps. This accounts for loops with frilled edges not infrequently met with, particularly in straps (Pl. I, F).

SPORE CULTURES

Series 1.

Started 21st. April with swarmers from Salt Vlei Bay material. Final observations 14 October.

When liberation of swarmers was obtained, clean slides were placed slightly sloping against the sides of tumblers containing sea water in which were swarmers already liberated or pieces of thallus in process of liberation. Swarmers collected in numbers on the slides, settled down and rounded off. The following day the slides were transferred to fresh filtered sea water, reversed so that the surface with the spore was turned outwards.

For some days no sign of germination was seen and when it finally began, it was slow and soon arrested. By the 11th. May elongation had begun in many of the spores.

The slides were washed and transferred to fresh seawater but now a few drops of Schreiber's culture solution¹⁾ were added. The addition of this nutrient solution had an immediate effect, stimulating germination and growth.

When the minute green planozygote settles down it withdraws its flagella and secretes a wall (fig. 2, N), then begins to enlarge, the stigmas persisting for some time even after germination has begun (fig. 2 N-a). It begins to enlarge, the contents becoming bright green and usually granular. Elongation follows and soon a rhizoidal out-growth forms on one side of the spore becoming paler green as it develops (fig. 3, A-C). This stage had been reached in untreated seawater but there development ceased; when Schreiber's solution was added growth was resumed. A transverse septum cuts off the narrower rhizoidal initial from the upper cell, which is wider with richer green content (fig. 3, D). The upper cell enlarges and a succession of transverse divisions soon results in a row of cells. The end cell of the filament forms a dome shaped cell rather larger than the other cells of the filament which for a time functions as an apical cell, segments being cut off parallel to its base (fig. 3, E, J, K). The basal cell either produces an elongated nearly colourless rhizoid, green in its upper part (fig. 3, D, E) or instead of elongating forms a broad polyhedral cell firmly attached to the slide (fig. 3, O, P). In the former case the primary rhizoid is usually soon supplemented by secondary rhizoids arising either from the upper part of the rhizoid-initial cell, or from the basal cells of the filament (fig. 3, F, G, K). If

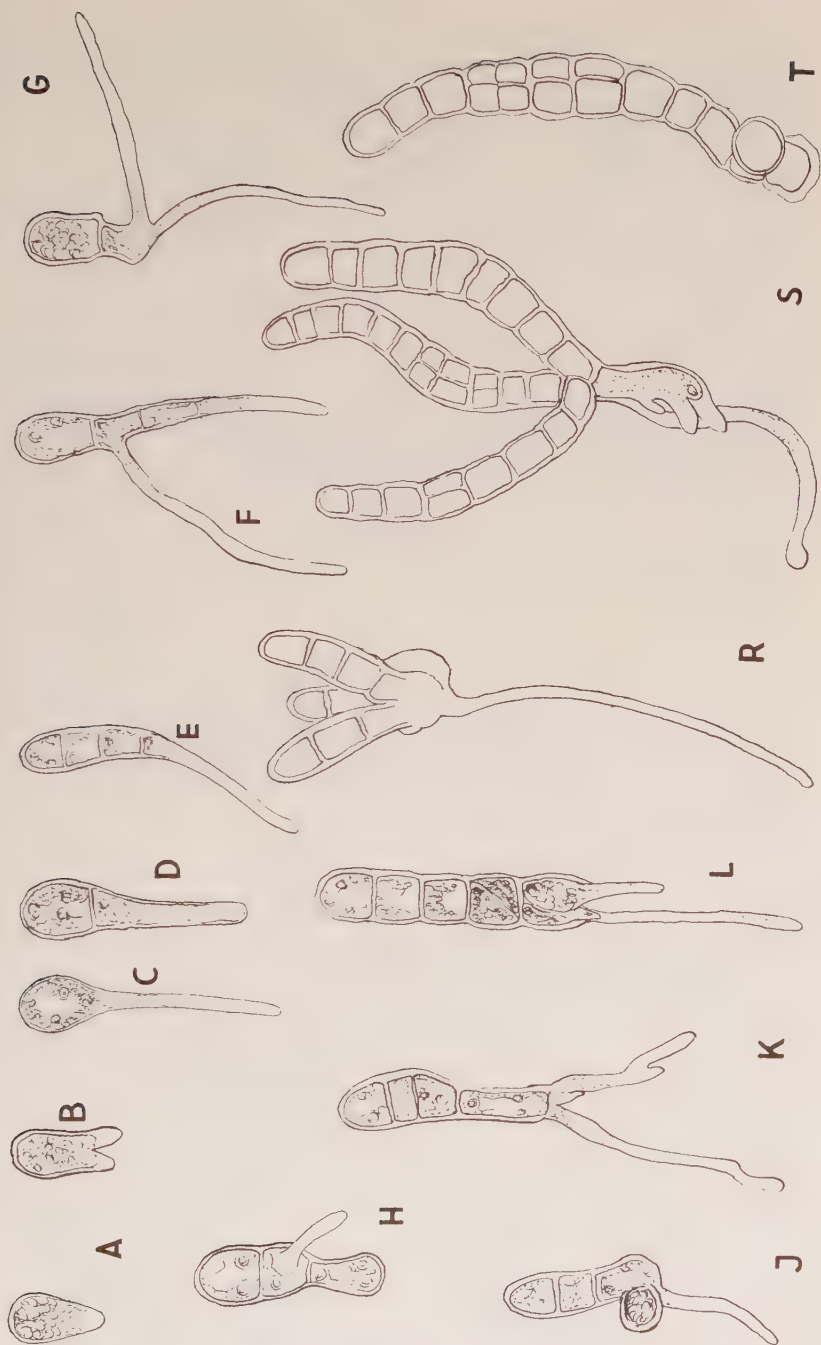
¹⁾ SCHREIBER 1930. The solution is made up as follows: - NaNO_3 0.1 gr., Na_2HPO_4 0.02 gr.; Distilled water 50.0 cc., Seawater 1000 cc. See PAPENFUSS (1942).

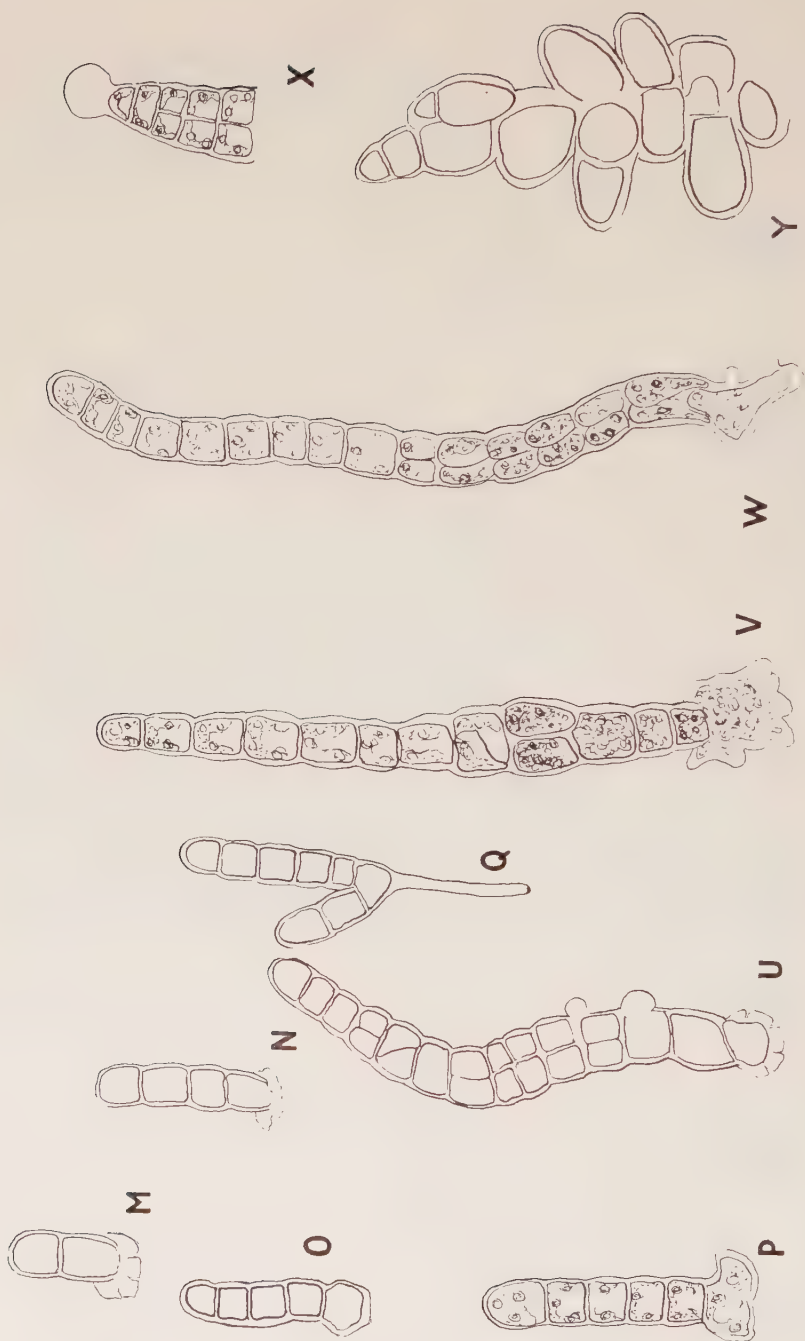
Figure 3

Germination: A-C, elongation: A, B, initial stage, in B, the rhizoid initial divided; C, rhizoid well developed, green in upper part; D-E, transverse division beginning: D, first division cutting off rhizoid initial from initial cell of filament; E, filament three celled. F-L, various forms of development of rhizoid: F, H, secondary rhizoid formed from second cell of filament in F, from first cell in H; G, K rhizoid branching to form two rhizoids; L, rhizoid initial cell divided longitudinally, each daughter cell producing a rhizoid. M-P young sporelings with polygonal basal cell. J, Q-S, young sporelings showing branching of the filament, each branch initiates a frond: J, three celled filament with bud of branch at base, in Q secondary branch already 2-celled; R, sporelings with 3 branches each 3-celled: S, older sporeling with longitudinal division starting in central region of 2 branches, third branch broad but still monosiphonous throughout. T-W, stages in development of unbranched filament: T, longitudinal division just beginning, branch initial from lowest cell of filament; U, longitudinal division well advanced, 2 spherules of callus on side of filament; V, W, showing granular lower cells, upper cells with parietal chloroplast; in W daughter cells of lowest cell of filament producing rhizoids growing down over basal cell; in all four hyaline halo of callus round foot of basal cell well developed. X, apex of older filament, two cells undivided, bubble of callus on apical cell. Y, proliferation and blown up cells.

Figures A, L, M, N, T, U, X, from Waterloo Bay cultures, the rest from Salt Vlei.

All $\times 340$ approximately.





however a polyhedral cell is formed, the wall soon begins to show hyaline thickening suggesting the initiation of the holdfast by secretion of callus (fig. 3, V, W). As this develops it forms an adhesive pad gluing the basal cell to the slide. In some cases rhizoids as well as the polyhedral cell developed (fig. 3, H, S). After transverse divisions have produced a filament of some 6 to 10 cells longitudinal divisions begin starting usually not in the lowest cell but some cells above it, then spreading down to the basal cell (i.e. the cell above the rhizoidal cell) and up towards the top of the filament (fig. 3, S, V, W).

Soon the germling has the appearance of a solid rod, two cells in width except for the rhizoidal initial cell and for a varying number of cells at the apex which still remains monosiphonous, the apical cell adding to the length by cutting off segments parallel to its base. From the two daughter cells of the basal cell rhizoidal outgrowths are sometimes put out, making their way downwards over the rhizoidal initial cell (fig. 3, W). Sometimes at a very early stage a filament began to put out erect branches near its base (fig. 3, Q-S), while in other cases a cluster of spores grew into filaments so inextricably intermingled that they formed a single plantlet with a common attachment organ.

At this stage it became obvious that the position of the slides, resulting in the sporelings being on the lower sloping surface, was unsuitable since the plantlets could not grow erect. Instead of assuming the normal erect position in their efforts to do so they were forced to grow creeping over the surface of the slide and a number of hyaline secondary attachment organs began to appear here and there on the filaments. In addition the slides were badly contaminated with diatoms, ciliates, amoebae and some minute organism, possibly bacterial, which attacked many of the spores, mostly ungerminated ones the empty walls of which remained on the slides among the germlings. Consequently growth was becoming abnormal; parts of the plants, particularly the monosiphonous apical portions were degenerating. When the cultures were finally discarded on the 14th. October many of the plantlets were still green and developing but in a curious way: there was still no sign of a flattened expanded frond, but the cells were beginning to grow out at right angles to the surface of the frond, sometimes as enlarged rounded cells, sometimes forming monosiphonous filaments attached to the slide here and there by hyaline pads of callus secreted by the wall of the cells (fig. 3, Y).

Series 2.

Started 2nd. July 1958 with swarmers from Waterloo Bay.

As already described, liberation had occurred in vast quantities at

about 11 o'clock in the morning; water green with swarmers was collected and brought back to Grahamstown. When examined at 4 p.m. of the same afternoon the swarmers were found to be still actively motile and many were still motile the following morning. The majority were quadriflagellate, though biflagellate swarmers were also present. In some of the former two stigmas could be distinguished but to begin with the stigmas were not very well differentiated. Collected either on slides and coverslips lying flat on the bottom of dishes or on slides sloped against the sides of tumblers as in the previous cultures many settled down and the following day the slides etc. were transferred to fresh sea water plus Schreiber's solution, some standing, others lying flat. From time to time the slides were washed and transferred to fresh culture solution -always filtered sea water with Schreiber's solution added.

On the 4th. July the spores, which had settled down very thickly, already showed signs of germination. In many, two eyespots, much more distinct than when first examined two days earlier, could be seen both in still rounded spores (some considerably enlarged) and in others which had already begun to elongate (fig. 2, N-P). The spores now showed considerable variation in size, from $4.5\ \mu$ to $7.2\ \mu$, each with a single parietal chloroplast partly filling the cell, and two eyespots lying sometimes side by side, sometimes on opposite sides of the cell. Germinating zygotes were already considerably larger ($9 \times 13\ \mu$), with several pyrenoids (figs. 2, O-Q). Unfortunately few survived - whether, as might be expected, because viability had been too far lowered by the long period intervening between liberation and collection on the slides, with accompanying high temperature in the car, or because contamination became very bad, or from a combination of the two, is uncertain. The parasite which had been troublesome in the Salt Vlei Bay cultures was even worse here, destroying the contents of the spores so that a great part of the slide was covered with empty spore walls. Later, besides the innumerable diatoms, other algae developed. On one of the few slides which survived on the 20th. September there were a few healthy sporelings; the surface of the slide was mostly covered with diatoms including a branched filamentous species in addition to numerous single cells, while here and there were scattered filamentous Green algae, one prostrate, another forming small bushy plants. The same filamentous Brown alga which occurs in holdfasts and warts, here too formed bushy little clumps; patches of a coccoid Blue-Green alga giving an intense grey-blue colour occurred at several spots, while most surprisingly there were two well developed female gametophytes of *Ecklonia biruncinata* with young sporophytes.

In most of the sporelings the basal cell was polygonal, surrounded

by a hyaline pad of callus gluing it firmly to the slide (fig. 3, M, N); some sporelings were still a single row of cells, others, one $172\ \mu$ in length showed longitudinal divisions beginning near the centre of the filament (fig. 3, T, U). By the 14th. October, however, most of the sporelings had started proliferating like the Salt Vlei material, the much enlarged cells growing out from the surface, sometimes forming filaments of several cells.

The cover-slip cultures, placed singly on the bottom of small pyrex culture dishes, developed rather slowly but proved of considerable interest. Filtered sea water plus a few drops of Schreiber's solution was again used, the slips washed from time to time and the water changed. At first rather too much Schreiber's solution was added, resulting in very congested granular cells. Later the culture solution was changed more frequently (every third or fourth day), freshly collected filtered sea water was used, the bottle containing it being well shaken to aerate it before the culture dishes were filled and the Schreiber's solution was reduced to three or four drops. Later growths were less congested and more normal in appearance.

The two cultures proved very different from one another. On the first coverslip, A, the zygotes had settled down very thickly, contamination was less than in any other culture and the majority of the spores germinated, resulting in a thick turf of sporelings. On the 20th. September most of the sporelings were growing erect showing all stages of development from two- to many-celled filaments (fig. 3, M, N), many with one or two branches arising from the base, the apical cell dome-shaped, and in most the basal cell truncated with a polygonal base surrounded by a hyaline halo. In a few however long rhizoids had developed. The erect position made it easy to see the attachment pad, but more difficult to study the structure of the erect filaments since they were still too short to be flattened by a coverslip.

By the 14 October the sporelings, markedly phototactic, though still short, had developed considerably, most of them little bushy plantlets with several branches, the majority still a single row of cells, but some showing longitudinal divisions near the centre of the filament.

By the end of the month the bright green turf, viewed sideways, was of appreciable thickness; it was interrupted by five dark brown spots which were bushy growths of the filamentous Brown alga already described, with fainter brown patches here and there where younger plants were developing, sporelings growing intermingled with the Brown alga. Diatoms were comparatively few, mostly single but one or two plants of a filamentous species. Colourless protista were present, a few ciliates and occasional unicellular Green algae, but all sparse and few compared with the other Waterloo Bay cultures.

On the 1st. November the sporelings were all bushy, the cells in the lower parts congested with dark green granular chloroplasts. In some cases the primary filament was more than two cells wide and in a few of these rhizoid formation from the lower cells had begun, the rhizoids growing down between the cells and emerging near the base to supplement the primary holdfast. In some cases proliferation had started; some of the proliferating cells were blown up but most of them were giving rise to short branches. Most of the latter were still monosiphonous but in others longitudinal divisions had started. The lower cells of the branches still showed granular chloroplasts, but in the upper cells the chloroplasts were becoming more homogeneous, parietal with several pyrenoids.

Where the holdfast could be seen it was well developed with considerable secretions of callus and in many cases with long pale green rhizoids growing out from the base. Occasional hyaline bubbles of callus had been formed either on the apical cell (fig. 3, X) or laterally on the filament (fig. 3, U).

After a few minutes under a cover slip, the surface of the culture was covered with minute bubbles of gas, presumably oxygen given off by the algae, synthesizing actively in the strong light of the microscope.

The experiment was carried on, but on the 26th. November little progress could be seen. Contamination by diatoms was now very bad in the outer part of the cover slip, where the larger plants were scattered and many of the filaments had their tips (apparently the most vulnerable part) covered with diatoms and had made little progress. One case was interesting: the apex of six undivided cells was dead and had been cut off by a dome shaped wall behind which a multicellular apex had developed. Apart from this the apices were all monosiphonous several of the apical cells with bubbles of callus. In the centre of the inoculation the plantlets were thickly crowded and though less badly attacked by diatoms had made no appreciable progress, probably due to over crowding. Many of the branches were monosiphonous throughout or had only a few of the central cells divided longitudinally. Cells in the lower parts of the filaments were congested and granular and many of the plants were developing blown up cells and proliferation near their bases. Holdfasts were mostly flat pads, in a few cases with long rhizoids.

The second cover slip, B, was a complete contrast. It had been overlooked, then on the 19th. October was found to be so brown with diatoms that it was about to be discarded. But on examination it was discovered that, scattered here and there, were well developed *Letterstedtia*-sporelings. In most of them the basal parts were similar to

those in other cultures, with granular chloroplasts, but arising from these were erect branches very different in appearance, the colour paler green, the cells not congested except towards the base, and actively dividing. One plantlet, (a), consisted of a little bunch of six or more branches of varying lengths; throughout most of their length the cells in these branches had divided longitudinally, the number of undivided cells in the apical region ranging from one in the longest ($630\ \mu$) to as many as nine in the second longest. Two other plantlets, (b) and (c), each with a single erect branch $840\ \mu$ and $1050\ \mu$ respectively, arising from a short basal portion, showed the beginning of broadening, being more than two cells wide. The lower cells were granular and dark green, but in most of the cells the chloroplasts were changing from the granular type to the more homogeneous-appearing parietal chloroplast with a few pyrenoids which is characteristic of the adult plant. In some of the older basal regions there were signs of proliferation, but most of the proliferating cells were giving rise to additional erect branches.

The cover slip was washed and transferred to fresh filtered sea water, aerated by shaking the bottle well, plus three drops of Schreiber's solution; this culture medium was now changed at intervals of from two to four days.

On the 31st. October the length of the longest branch of (a) had increased to $1059\ \mu$; it was now multicellular throughout with a broadly rounded apical meristem (fig. 4, C, E). Plant (b) measured $1501\ \mu$ and (c) $1680\ \mu$, both several cells wide in their widest part.

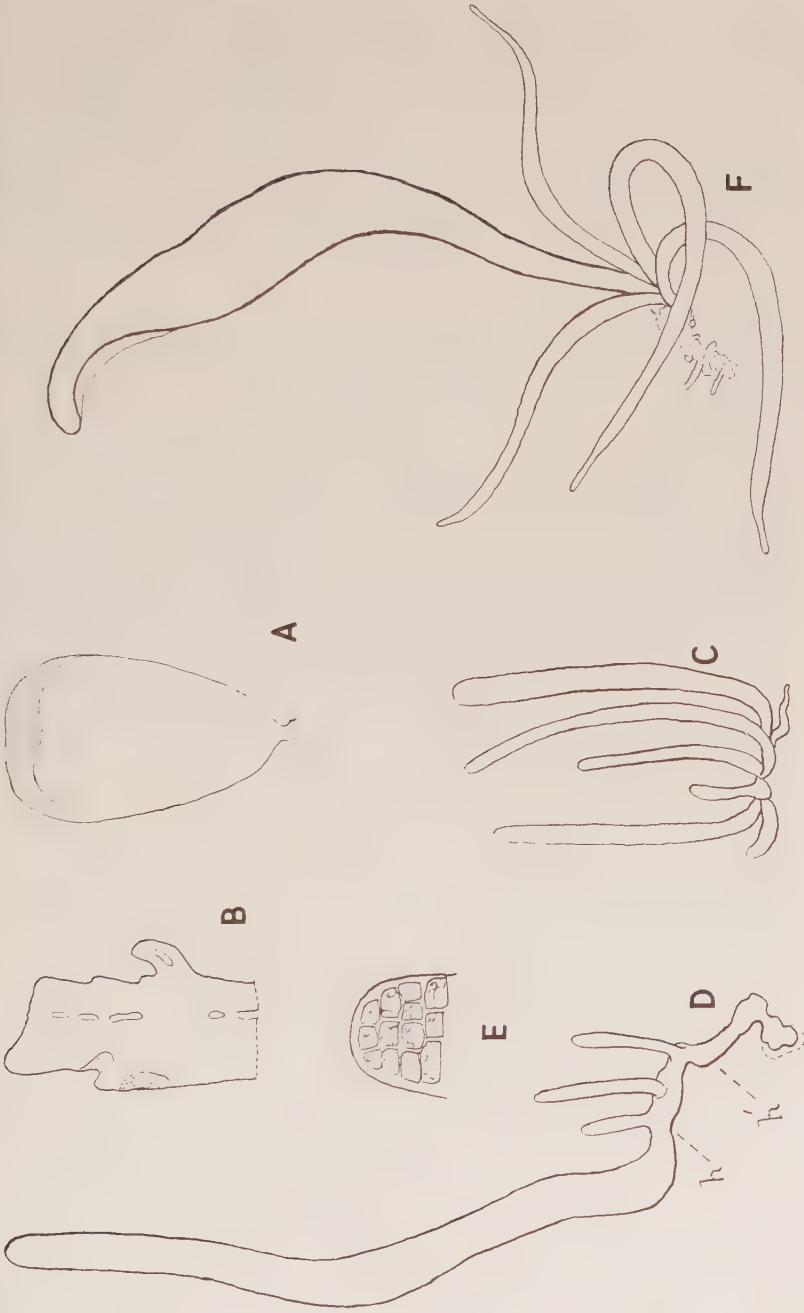
The contaminants in this case differed in some respects from those in other cultures. Diatoms of course were abundant, covering the cover-slip with a continuous brown film; occasionally a filamentous species was present but most were single cells. In addition there were three types of unicellular Green algae, one forming sheets of minute rounded cells less than $3\ \mu$ in diameter, the second ($4 \times 6\ \mu$ to $8 \times 10\ \mu$) somewhat egg shaped with two or more chloroplasts without pyrenoids, the third larger spherical cells, $11\text{--}13\ \mu$ in diameter, with a conspicuous pyrenoid – in fact, very similar in appearance to *Letterstedtia* zygospores. Unfortunately the washing of the cover-slip proved to have been a mistake since it had disturbed the diatoms, many of which were now attached to the erect filaments of the sporelings.

On the 3rd. November some of the sporelings were covered with diatoms; others however, taller and growing erect, were still comparatively free and healthy. Plant (a) was badly infected, some branches, including the longest, too thickly covered to be examined, but one branch still erect and fairly clean measured $882\ \mu$ and was multicellular throughout its length, including the apex; several small

Figure 4.

A, young sporeling with apical area fertile. B, young frond with median row of holes left by evacuated fertile areas, two marginal fertile areas *in situ*. C, plant (a) from cover-slip culture. D, plant (c) from same culture, erect filament several cells wide, apical meristem formed. E, multicellular apex of longest frond in plant (a) and main filament of (c). F, plantlet with several branches, primary frond beginning to flatten.

A, C \times 8; C \times 40; D, F \times 50; E \times 400



branches were developing at the base of the plantlet. Plants (b) and (c) had increased to 1890 μ and 2000 μ and in them too the apical cell had divided longitudinally to form an apical meristem (fig. 4, D, E). A couple of weeks later (14th. November) (b) measured 1953 μ and (c) 2373 μ long by 73 μ wide. On the 24th. November (b) was 2142 μ long and (c) 2520 μ long by 77 μ wide. Plantlet (a) was too covered with diatoms for exact observation – it could just be seen that two of its branches had multicellular apices, the rest being still monosiphonous at the tips.

Plant (c) was particularly interesting. It was the largest and healthiest sporeling yet obtained in any culture; just above the holdfast a short length was horizontal, lying on the cover-slip; from this part, the three branches, which had helped to identify the little plant throughout the experiment, had arisen. All three were now well advanced with longitudinal divisions in most of their length, while between them and the right angled bend which brought the long filament erect, several new branches were arising. In handling the coverslip the whole plant was turned over tearing the diatomaceous film loose on one side. Turned on edge, this revealed that two secondary attachment organs had developed at h and h' in the horizontal portion (fig. 4, D). Each consisted of a fairly massive greenish outgrowth penetrating through the diatomaceous film and giving rise to slender colourless rhizoids growing along the surface of the glass.

Contamination had increased considerably; besides the diatoms, the ciliate, obviously feeding on the smaller diatoms, was now very abundant as was the small colourless flagellate.

When finally examined on the 6th. December plant (c) had not recovered its erect position properly and had a good many diatoms on it; its length had increased a little (to 2730 μ), but it had not increased in width at all.

But near the edge of the cover slip several plants had begun to develop well, and in one of these, at last, flattening was beginning in one branch, rather irregularly but quite unmistakably. The main branch was 1890 μ in length and 138 μ wide in the upper spatulate portion, which was about seven cells wide with parietal chloroplasts. In the lower part the cells were congested with granular chloroplasts which were being replaced towards the base of the spatulate portion by the more homogeneous parietal chloroplast with two to four pyrenoids characteristic of mature cells. From the base of this main branch four shorter branches spread out, all with long monosiphonous tips (one with as many as 14 undivided cells in the apical region), but the greater part of each divided longitudinally, again the lower cells with granular chloroplasts, the upper with the normal parietal chloroplast (fig. 4, F).

SPORELINGS GROWN IN NATURAL CONDITIONS

In all these spore cultures certain phenomena suggested abnormal development. Chief among these were: – 1) Slowness in development, since field observations indicate that growth is probably normally rapid. 2) Congested granular appearance of enlarging spores and of cells in the young filaments. 3) „Blown up” cells and proliferation of the cells of many sporelings. 4) Absence of flattening of the filament to form a thalloid frond.

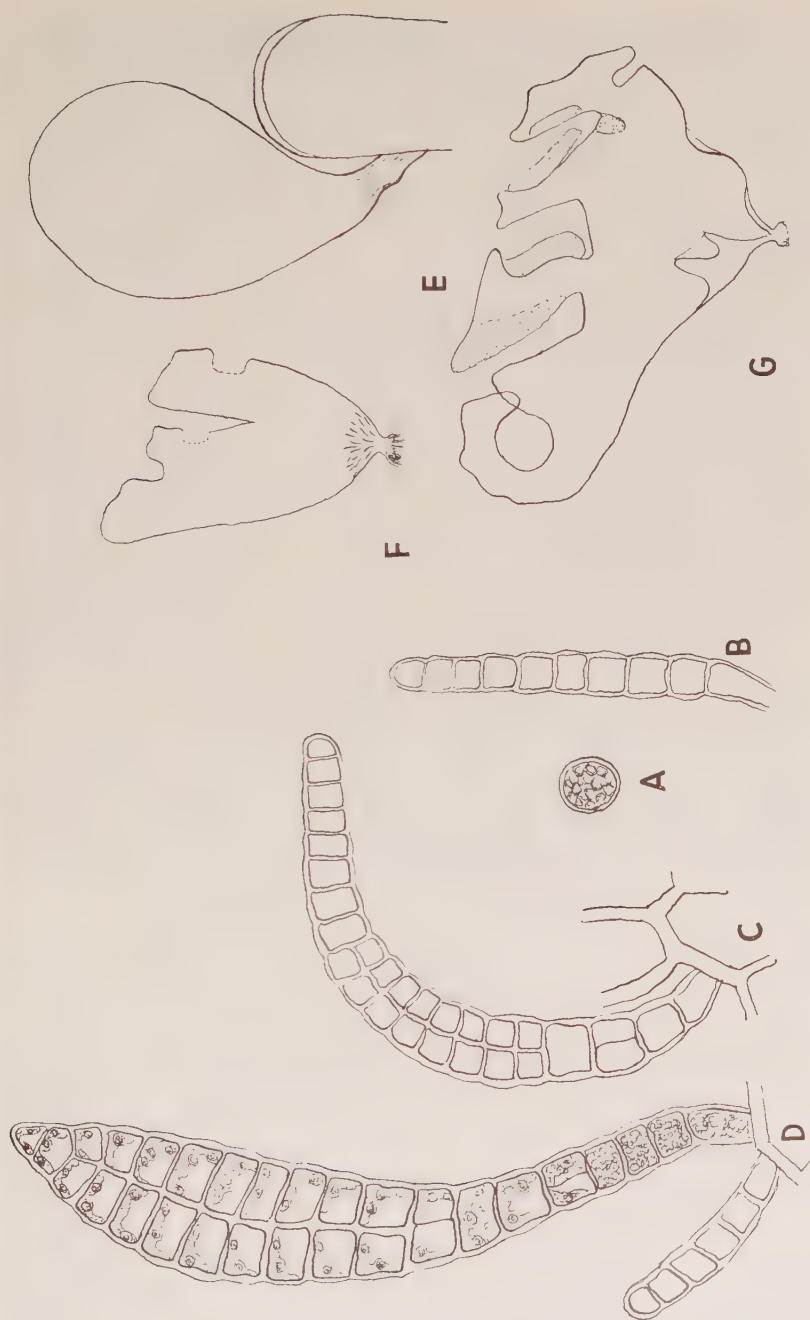
Fortunately, on the 28th. October material brought in from Salt Vlei Bay provided a criterion against which to compare the sporelings obtained in culture. Several well developed sporelings on *Codium Duthieae* were collected and while examining these, many younger sporelings at different stages of development were found. As a rule these sporelings were not attached directly to the *Codium* utricles (though in one instance this was apparently so – fig. 5, E) but to *Placophora Binderi* epiphytic on the *Codium*. Several pieces of *Placophora* were detached from the host and one of these was found to have a remarkably complete range of stages in developing sporelings. These included 1) One or two spores still in process of enlarging. As in the cultures these were deep green with granular chloroplasts. 2) Very young filaments consisting of a single row of some 6 to 10 cells. 3) Older filaments showing cells in the central region divided longitudinally. 4) Yet older filaments where flattening was beginning and the sporeling had become spatulate. And finally 5) Several sporelings of various ages, already thalloid, some fertile.

From these and other sporelings found it seems obvious that a) the deep green granular appearance is normal in enlarging spores and in the basal cells of young sporelings but is accentuated when too much Schreiber's solution is used. b) As development proceeds the granular structure disappears, the chloroplast gradually becomes homogeneous, at first with numerous pyrenoids which decrease in number as the cells assume the normal size and structure, until the single parietal chloroplast with two to four pyrenoids lines the upper part of the cell as in normal meristematic cells. Again, continuance of the congested granular appearance in cells of the filament is attributable, in part at least, to too free use of the supplementary culture solution. c) The early stages in development, enlargement of the spore, followed by elongation and finally the appearance of first one, then successive transverse septa to form a monosiphonous filament with a specialized basal cell, forming an attachment organ: growth in length by the activity of a dome-shaped apical cell; appearance of longitudinal divisions, first near the centre of the young filament, thence spreading downwards to the second cell from the base and

Figure 5.

Sporelings from *Placophora* epiphytic on *Codium Duthieae*: A, enlarging spore. B-D stages in development of the filament, all still with dome-shaped apical cell; B, and smaller filament in D still monosiphonous; C, longitudinal division well advanced in central part of filament; larger frond in D flattened, spatulate although only two cells wide; lower cells with granular contents, upper with parietal chloroplasts. In all, base hidden in tissue of *Placophora*. E, young thalloid sporeling on *Codium*, F, G, older sporelings, G with three eroded places on the margin where fertile areas have sloughed off, G, with similar eroded margins and five evacuated fertile areas still *in situ*, one beginning to slough off.

A, $\times 840$; B-D $\times 336$; E, $\times 25$; F, G. $\times 8$



upwards until only a few of the apical cells remain undivided: all appear to be the normal sequence of development, the development of the basal cell varying considerably according to the nature of the substratum. d) Extension of longitudinal divisions to the apical cell itself, so that the single dome-shaped cell is replaced by a meristem of small cells appeared late in development in the cultures, while flattening of the sporeling to form a thallus was seen only in the one instance described above; probably in normal development these two changes accompany one another, the change in the apex occurring when the young plant finally assumes the thalloid form. In the cultures what broadening of the filament and subsequent flattening were obtained was accompanied by an increase in the number of cells without increase in their width. In the spatulate sporeling (fig. 5, D) on *Placophora* widening of the sporeling was brought about by a marked widening of the cells still only two in number across the thallus, and the apical cell was still undivided. Unfortunately the stages intermediate between this condition and the assumption of the typical thallus-form, with a number of small cells forming the width of the thallus, have not yet been seen. With improved culture conditions it is more than probable that the sporelings could be carried much further in development, the full sequence of changes from the spore to the young thallus appearing normally.

In the sporelings on *Placophora* it was difficult to distinguish the attachment organs as they were buried in the tissue of the Red alga. So far as could be seen they consisted almost entirely of rhizoidal growths with very little development of callus. Further study of such sporelings however would probably add considerably to our understanding of the course of development in *Letterstedtia*.

DISCUSSION

1. External Morphology.

The original and all subsequent descriptions of *Letterstedtia insignis* convey a very inadequate idea of the complex and extremely varied form of the plant. Herbarium specimens are seldom entire plants and even when they are, one specimen can give only a partial picture of the possible variations. Further, nearly all such specimens are plants or parts of plants showing the deep water, summer form, with well developed straps. The commoner bushy type of plant, characteristic of shallower water and found throughout the year, has been completely overlooked and is consistently ignored in all descriptions of the alga.

A well developed adult plant growing completely submerged even

at low water consists typically of a more or less bushy basal part with the characteristically elongated narrow straps growing from its upper edge; plants with narrow strap-shaped basal fronds also occur, but are less typical. At the other extreme, characteristic of shallower water and found throughout the year, are bushy plants often with deeply dissected fronds and irregular crimped margins, not unlike a robust crimped *Ulva* in appearance and probably usually mistaken for one. In between these two extremes are found plants showing every gradation from one to the other.

An analysis of the parts of the plant shows that from the primary frond appendages of various types arise as outgrowths of the margins. These include lateral fronds, leaflets of various shapes, narrow elongated straps, all varying considerably in position, shape and size.

The form of the primary frond itself shows great variability, partly correlated with position, seasonal growth, depth of water, etc., partly with differences in the primary form and later development of the plant. In shallow water it may be a broad expanded structure, its margins more or less cut up and thrown into folds, while in deep water summer plants where growth is rapid it may take the form of a narrow strap-shaped structure, bearing marginal proliferations. It may be split into two or more portions almost to its base, or a single plant may consist of several distinct fronds originating as branches arising within the holdfast either in the sporeling stage or later, each repeating the varied development shown by the primary frond.

In the sporeling there is a short but distinct terete stipe, but very early in development this disappears entirely in the large conical attachment organ so that the flattened fronds emerge directly from the apex of the holdfast (fig. 1, D).

Lacination of young fronds originating in the formations of lines of median fertile areas may contribute to the initial dissection of such fronds but in this species plays only a minor rôle in the evolution of the complexity of the adult plant.

The contrasting shades of green are very striking; all young parts, especially the frills, are a vivid dark grass-green, the central region of the straps a paler rather yellower green, while the older parts of the frond are intermediate in colour.

2. Anatomy.

In considering the complexities of external form the internal anatomy must inevitably come into the picture since the two are obviously closely interrelated. All young parts – young basal fronds, margins of the adult frond, leaflets, frills of the straps or broader fronds – are composed of relatively thin walled cells which retain

their primary structure and with that the power of division and of reproduction, that is, they are meristematic. Their bright green colour easily distinguishes them from the paler green permanent tissue.

It is in this differentiation into meristematic and permanent tissue, found in all parts of the plant, that the clue to the complex external morphology must be sought. The presence of a persistent diffused marginal meristem in all parts of the plant enables it to produce at any stage and in any part appendages of very varied forms, allowing for a high degree of complexity and almost infinite variation in the external form of the plant. A comparison of the photographs reproduced in Plate I will give some idea of the range of this variability.

The power of growth retained by the young parts is remarkable; even at the extreme base of the frond young ribbon shaped fronds or leaflets may grow out; the more specialized narrow margin of the straps by its rapid growth is thrown into folds to form the characteristic plicate frill and this too may produce lateral appendages. Combined with the formation of reproductive areas, often overlapping, sloughed off after evacuation of the swarmer, marginal growth results in continually changing contours, eroded margins, truncated tips to leaflets and young fronds often leading to a form of dichotomy, etc., while splitting of the thallus from various causes may also contribute.

But however complex the form of the plant, anatomically the basic structure remains the same throughout, referable to the ulvacean type of construction, that is, the whole plant is thalloid, built up of two inversely orientated layers of cells, elongated at right angles to the surface and enveloped in a common wall. Probably *Letterstedtia insignis* represents the highest degree of complexity to which this type of construction is capable of attaining, this being made possible by two factors: – 1) the differentiation into meristematic and permanent tissue combined with 2) the apparently unique type of secondary growth in thickness.

The primary structure, however, differs from that of *Ulva*¹⁾ in one particular, apparently insignificant but actually of far-reaching importance, that is, in the close juxtaposition and partial fusion of the inner walls of the cells of the two layers. Even in the young meristematic parts the contact between the inner walls exists, but as the tissue passes over into the permanent state union becomes progressively more complete, and continual addition of wall substance to the fused walls leads to the building up of the complex system of

¹⁾ The only exception to the construction accepted as typical of *Ulva* that the author has found in the literature is a figure by CHIN-CHIN JAO (see TAYLOR, 1937 Pl. 4, fig. 9) of a transverse section of the thallus of *Ulva Lactuca* var. *latissima* where the structure is shown as like that of *Letterstedtia*.

trabeculae, which seems to be unique, comparable in some degree only to the trabeculae of *Caulerpa*.

Combined with the formation of trabeculae, outgrowths from the inner ends of those cells which are not concerned in the formation of the trabeculae result in the production of rhizoids which grow downwards, great masses of rhizoids packing the interstices of the trabecular system. The whole of this secondary growth results in an extraordinarily efficient structure both thickening and strengthening the thallus, found in any part of the plant, even the most extreme distal portions, and well adapted for life in moving water, often exposed to strong wave action. In heavy weather the straps may get twisted spirally and tangled but even the narrowest are capable of resisting a very strong pull without breaking.

3. The Wall Substance.

The chemical composition of the substances forming the wall of the thallus, walls of individual cells, the trabeculae, walls of the rhizoids, and callus wherever it occurs, needs investigating. The range of colour obtained on staining with iodine suggests that not one but several substances, differing slightly in chemical composition and easily transformed one into the other, are involved, possibly a complex of celluloses or hemicelluloses. Apparently the end product is reached in those parts which give the bright yellow stain, so reminiscent of the lignin reaction to iodine obtained in many vascular plants. Probably it resembles lignin in its properties of strength and elasticity.

The very thick walls and small empty lumen of the rhizoids, again, suggest the fibres of higher plants and undoubtedly they perform much the same function. The combination of rhizoids and trabeculae results in a very efficient system of strengthening and thickening of the thallus.

4. Distribution of the Thickening.

From the account given above it is obvious that the distribution of thickening varies not only according to the form of that part of the thallus in which it occurs, but also as a result of external conditions. Strength and flexibility depend partly on the amount of thickening, partly on its distribution. Thus, in long narrow straps where the main desiderata are flexibility and tensile strength, thickening is most often concentrated in a single midrib. At the other extreme, in the base of a broad frond, which has to some extent to support the plant, a more robust construction with some rigidity is needed, and a wide zone of thickening extends across the full width of the frond, often reinforced further by external se-

cretions of callus. Between these two extremes many intermediate stages may be found.

Again, correlation between the degree and distribution of thickening and external conditions may be instanced by reference to the delicate strap-shaped base of plants growing partly buried in sand. Here although the strap may be comparatively wide, thickening is reduced to a minimum – the fused inner walls are only slightly elongated and thickened and there are no rhizoids. But since the thickening process is a progressive one, should conditions alter, the thickening in such a region could develop further.

The case of narrow petiolar straps is interesting. In these the thickening is often spread over the whole of the very narrow central zone. The probable effect of such a structure would be to keep the petiole flat, thus tending to hold the leaflet away from the main strap and more or less in the same plane.

5. The Attachement Organ.

Here again *Letterstedtia insignis* shows marked individuality. Primarily, as in other Ulvaceae, the attachment organ is composed of a concentration of rhizoidal filaments pushing apart the two cell layers and emerging at the base to form an adhesive pad on the substratum. But here the rhizoids are reinforced almost from the first by the secretion of wall substance, internally partly in the form of trabeculae, partly amorphous cementing the rhizoids together, and externally in the form of conchoidal masses of callus building up the wart-like structure which soon envelopes the whole of the primary attachment organ and the stipe, entirely obliterating the latter. Judging from the adhesion of particles of sand or silt to the surfaces of the masses of callus these must be mucilaginous, although no other part of the plant shows any sign of a surface pectic layer.

That the nature of the holdfast depends to some extent on the kind of substratum becomes obvious from a comparison of sporelings growing on such varied substances as rock, straps and *Codium*, the proportion of callus to rhizoids varying considerably in different situations. On rock, callus soon predominates, typically sand-impregnated, the base flat or following the irregularities of the rock. On straps, especially on the edge of the frill, there is at first a predominance of rhizoid-formation the mass of rhizoids sometimes wrapping round the edge of the frill (Pl II, A), but soon the whole is enveloped in callus, usually almost free of sand, at any rate in the early stages. On *Codium* rhizoids predominated and very little callus was formed, but as only young sporelings were investigated it is quite possible that in later stages callus would again be produced in quantity.

In the spore cultures the position appeared to be of importance in determining the form of the young attachment organ. Sporelings growing on sloping slides most often produced rhizoidal attachment organs, individual rhizoids sometimes developing walls thickened with callus. Sporelings grown on cover-slips or slides lying flat on the bottom of the culture vessel nearly all developed pads of callus, sometimes reinforced by rhizoids from cells at the base of the filament. Here again only immature plants were studied and it is probable that eventually in every case a warty attachment organ would be formed.

The filamentous Brown alga so common in the substance or on the surface of warts has not yet been identified. What its effect, if any, on callus-formation may be is yet to be determined. The spore cultures show that it can also grow as an independent plant. The traces of animal organisms in warts are less general and in the attachment organ the association is probably fortuitous. Warts on the upper parts of the frond however, may perhaps be of the nature of galls produced in response to some external irritant. On the lower edge of the frond callus may be secreted as a result of contact-stimulus, forming secondary attachment organs. The bubbles of hyaline callus formed on many of the sporelings may be of the same nature, or possibly caused as a pathological response to unfavorable conditions in the culture.

But the universal occurrence of callus secretions in and about the holdfast proves that they are an integral part of the attachment organ, a structure perhaps peculiar to *Letterstedtia insignis*. Wherever it occurs, the substance forming the callus is similar in composition to that forming the trabeculae and inner layer of the common wall, giving a yellow stain with iodine.

7. Reproduction.

Since any young part with recently divided cells may become reproductive, in very young fronds or other parts in which differentiation of somatic tissue has not begun, reproductive areas may develop in any part of the thallus. But as differentiation becomes more extensive, meristematic tissue is confined to newly formed appendages and the margins of the older parts and reproductive zones are consequently mainly marginal.

When reproduction is proceeding actively it is usual to find areas at different stages of development overlapping one another. But fertile areas are always sharply delimited within two zones of non-reproductive, somewhat thick-walled cells. Thus each successive fertile area is cut off from its predecessor by one or two rows of cells which are not participating in reproduction, and as the evacuated area is

sloughed off these cells form the new margin of the frond, whether the adjacent tissue is already becoming fertile or not.

In *Ulva*, according to SMITH (1947 p. 82), the margin of a fertile frond shows a similar border of non-reproductive cells, but within it differentiation of fertile cells is progressive from the outside inwards until the fertile zone may be of considerable width.¹⁾

In *Letterstedtia insignis* even in the frills where a fertile zone may be several centimetres in length, its width is never more than a very few millimetres (1 to 3 mm. at the most) and as it sloughs off a border of non-reproductive cells protects the margin of the inner meristematic region. In other parts the fertile areas are always of limited extent, often in the form of small arcs or segments, or sometimes a longer marginal zone may be differentiated, but always sharply delimited, never progressive inwards. There may be a series of several such small areas overlapping – the outermost already evacuated and ready to be sloughed off with the incoming tide, the next with gametangia already divided and the gametes ready for the final change preparatory to liberation at the next low tide, then a zone in which division of the gametangia is just beginning, and finally meristematic cells recently divided, – each section with its outer margin of comparatively thick walled cells (c.f. Pl. II, G-H).

FRITSCH (1935 p. 216) states that the number of gametes in *Ulva* is usually eight. SMITH (1947 p. 82), working with the five Pacific species found on the Monterey Peninsula found considerable variation in number. All five species were heterothallic, all but one anisogamous. In *U. lobata*, which he studied most intensively, 8 or 16 zoospores were formed in each sporangium, while in the gametophyte generation 16 or 32 of the larger, ♀ gametes and 64 or 128 of the smaller, ♂ gametes were produced in each gametangium of the ♀ and ♂ gametophytes respectively (here again there is a slight discrepancy between this and his later account (1955 p. 63) where he gives 64 as the highest number of gametes per gametangium in this species.

In *Letterstedtia insignis* some variation in the size of the gametes was found and in at least one copulating pair, the two gametes were slightly different in size (fig. 2, H), but as yet there is not sufficient evidence to decide whether this species is isogamous or anisogamous. The number of gametes was very constantly 16, in only a few cases of larger gametangium were 32 gametes found here and there among those with the normal number of 16, and the size of the rounded

¹⁾ SMITH (l. c.) gives „10 to 15 cm.” inwards from the margin, but in his text book (1955 p. 63) the fertile zone is said to be from 5 to 15 mm. This suggests the possibility of a typographical error in the earlier account.

daughter protoplasts in mature gametangia was very uniform (diameter between 5 and 6 μ); in those cases where some of the contents of a gametangium were larger than the others they were always fewer in number and the larger bodies had either two or more eyespots, indicating that there had been fusion within the gametangium between some of the immature gametes. This occurred in fronds which had been kept for some time before examining them and is regarded as a pathological phenomenon, not normal conjugation within a gametangium.

Escape of the gametes has not actually been watched, but from the fact that they have been seen moving actively within the gametangium shortly before escape, it seems probable that liberation takes place in the motile state, and not, as SMITH found in the Monterey species of *Ulva*, while still non-motile.

Early in the investigation pieces of fertile fronds were found on which swimmers had settled down and rounded off. In this condition the eyespots are persistent, remaining conspicuous for several days, even after the differentiation of a wall. Some, presumably gametes which had not conjugated, had only one eyespot, while others, obviously zygotes had two. Cells of both types began to germinate, those with one eyespot rather more freely than the others. Unfortunately none got beyond the initial stage of elongation. Consequently the possibility of parthenogenetic development of gametes still remains unproved, though from such observations it seems at least possible. SMITH (1955 p. 63) quotes FOYN (1934) and MOEWUS (1938) as recording the occurrence of parthenogenetic development of gametes into gametophytes, but he himself found that, in *U. lobata* as in certain other species, gametes that had failed to conjugate soon disintegrated without further development.

The sloughing off of evacuated fertile areas soon after liberation of the gametes is a very characteristic feature. That it is largely mechanical as the result of wave action was well illustrated by the difference noticed when the *Letterstedtia* area was completely isolated from the open sea on the 2nd. June. Whereas normally colourless areas were rare in Salt Vlei Bay where even at low water the *Letterstedtia* zone was subject to wave action, on this occasion colourless rims and patches were common on nearly every plant examined. It would seem that normally the evacuated areas would all be stripped off before the next low tide.

8. Nature of the Swimmers.

The biflagellate swimmers are almost certainly gametes, which may perhaps be facultative, capable of development without conjugation, while the quadriflagellate swimmers are planozygotes since when-

ever they were found signs of conjugation were always present while in many of them two eyespots could be distinguished. Unfortunately, while sometimes the eyespots were easily distinguished, this was not always the case in the motile state; after the swimmers had settled down and rounded off the eyespots frequently became more distinct, persisting after the secretion of a wall and even in the early stages of germination.

Failure to find quadriflagellate zoospores may perhaps be attributable to a periodicity in the formation of gametes and zoospores comparable with that found by SMITH (1947 p. 85) in California. He found that in the first three or four days of a spring tide sequence gametes were formed. After the fourth day gamete production ceased abruptly, while a day or two later zoospores were formed. In each case the respective fruiting periods recurred at fortnightly intervals.

The observations on *Letterstedtia* were all made early in each spring tide and it is possibly due to this fact that zoospores have never been found. Unfortunately the distance of Grahamstown from the sea precluded the more frequent visits which were necessary; possibly later in the spring tide sequence zoospores may be formed here as in California. For the present, formation of zoospores remains an open question. The sub-littoral habitat of *Letterstedtia* adds to the difficulty of obtaining a complete picture of the sequence of events since at neap tide the plants are all completely submerged and mostly unreachable from the shore.

9. The Life Cycle.

It is thus obvious that knowledge of the life cycle of *Letterstedtia* is still incomplete. The possibility that the bushy plants represented one generation, the slender summer form the other was attractive and has been considered. But all the evidence is against this. Not only does every gradation between the two exist, but also swimmers obtained from young bushy plants and those from the large deep water plants have obviously been of the same nature, biflagellate gametes conjugating to form quadriflagellate planozygotes.

It is possible that further observations at other states of spring tides, as suggested above, may show that, as in *Ulva*, there is an isomorphic alternation of generations. As yet however there is no conclusive evidence either for or against this and the possibility that in *Letterstedtia* there is a different type of life cycle cannot be excluded.

From the nature of the sporelings obtained in culture it does not seem possible that the life cycle could resemble that of *Monostroma* where there is a conspicuous gametophyte and a small inconspicuous

sporophyte. But a third possibility, viz. that reduction division precedes gamete formation, though not perhaps very likely, is yet worth considering.

A cytological investigation of fertile plants and of sporelings obtained from germinating zygotes would no doubt provide a conclusive answer to the problem.

10. Spore Culture Experiments.

The spore culture experiments have been carried on under considerable difficulty, mainly lack of the time necessary for careful culture work and complete absence of any facilities for marine culturing. They have however been described here since incomplete as they are they indicate the possibility of such cultures in our alga and also confirm certain observations as to the composition of the plants. Some of the cultures have shown clearly that a *Letterstedtia* plant may consist of a single frond, or that branching from the base may result in a plant composed of two or more distinct fronds, or even that a single plant may actually be formed from a cluster of spores germinating in close juxtaposition. Comparison with sporelings developing *in situ* shows that the early stages of development obtained in culture are normal, and although the change from a filamentous form to a flattened thallus was only once obtained, development proceeded far enough to show the changes that take place in individual cells as the sporeling develops, including the change from a single dome shaped apical cell in the filamentous stage to a multicellular apex derived therefrom by repeated longitudinal divisions.

11. Duration and Seasonal Variation.

When this work began, it was held, mainly on evidence obtained from drift material, supplemented by the few collections of attached plants previously made, that there was a definite seasonal sequence, that „bushy” plants, as well as being characteristic of shallow water, represented the persistent winter form, while plants with long straps and usually less bushy bases were the deep-water and summer form of the alga. Up to a point this seems to be true but by no means does it tell the whole story. Bushy plants are certainly characteristic of shallow water or partially exposed situations and they do persist in winter, but they are found throughout the year and moreover, young plants are most often bushy to begin with, later developing straps. On the other hand, the well known large plants with long straps are characteristic of deeper water and are well developed in the summer months, but when *Letterstedtia* is abundant all gradations between

the two types may be found. Further, „summer” and „winter” cannot be taken in their strict sense.

In April 1958 at the beginning of the present series of observations tall plants with long straps were already well developed and abundant in Salt Vlei Bay. They continued in much the same state, perhaps even growing larger, in May and June, the early winter months, but by the beginning of August the straps were beginning to show signs of age, with few young leaflets, the central zone broader and with more epiphytes, while the frills had partially straightened out becoming less closely plicate. By October such plants had apparently completely disappeared, only bushy plants, many of them young, remaining. But on both visits in October conditions were so poor that an extensive search of the area could not be made and it is possible that some of the larger plants still survived in the deeper water. By the second visit in October even fewer *Letterstedtia* plants could be seen than earlier in the month, all small bushy plants, scattered here and there, while large plants of *Codium Duthieae* now dominated the area formerly filled with *Letterstedtia*. Unfortunately it had not been possible to visit Salt Vlei in September so that the exact date at which the disappearance of *Letterstedtia* had begun is not known. When next visited in mid- December, again conditions were unfavorable, much of the *Letterstedtia* area remaining submersed even at low tide, only the plants at the upper limit being even partially exposed between waves. All the plants were small and very young, often obviously a clump consisted of a number of sporelings, with a few slightly older bushy plants, very sparse but somewhat more abundant than in October. On the final visit, 25th. January 1959, however, although most of the area was still not fully exposed, there was a marked difference both in number of plants and their size and development. The population consisted entirely of bushy plants but most of them were developing straps while in the deeper pools foot-high plants had well developed frilled straps in the upper part. Nowhere, not even some way from the shore in 4 or 5 feet of water, were there any of the long „summer” type of plant which was such a conspicuous feature in May and June of the previous year. A few fertile fronds were noted.

At Waterloo Bay *Letterstedtia* is known to have been abundant in January 1958 although the writer knew nothing of it at the time and has unfortunately not seen any of the plants. Through the autumn and early winter months it continued abundant, always more bushy and with shorter straps than at Salt Vlei and always actively fertile. Even on the 14th. September, though much less abundant than formerly, there were still many plants with well developed straps, nearly all fertile. There had been much silting up of the pools caus-

ing considerable alteration in the aspect of the whole area. Young plants were fairly abundant among the older larger ones, while epiphytic sporelings, some of them quite large, were common. In January 1959, Mrs. RENNIE again reported it from Waterloo Bay and also from the bay to the east, at Impekweni, where it was growing on the rocks on both sides of the river mouth. There were many very young plants as well as larger bushy ones with developing straps. Still further east, it was seen wherever there are rocky outcrops as far eastward as the mouth of the Keiskama River. It seems probably that it occurs at intervals along the whole coast, although search for it in Natal by Mrs. JACOT-GUILLERMOD has as yet not been successful – probably on account of the state of the tide.

On the whole, the following seems to be a fairly accurate summary of the sequence of events throughout the year: –

Letterstedtia insignis first becomes noticeable in the early summer months (mid December or January) when bushy plants with developing straps are found; reproduction is general and the population is rapidly built up, reaching its zenith in May or June. It continues in profusion, large plants of rapid growth with very long straps developing in the deeper water while bushy plants predominate where it is shallower and partially exposed at low water, through the earlier winter months but by August the larger plants are beginning to show signs of age.

The rough weather typically occurring at or slightly after the September equinox shows its effect in a marked decrease in the population, the larger plants disappearing more or less completely; but by virtue of their lower stature some of the smaller bushy plants survive and with the advent of more favorable weather, the survivors become fertile and once again the population begins to increase.

In how far the disappearance of the larger plants is a natural phenomenon of age or due to weather conditions is not known. Probably both factors are operative in bringing about the changes in the population, and probably varying weather conditions may cause considerable shift one way or the other in the tentative timetable suggested here. Lastly, there is some evidence that a luxuriant growth such as that at Waterloo Bay in April 1942 and at Salt Vlei Bay in 1958 may be sporadic, that it may occur in one year while the following year the population, even at the height of its development, may drop to a much lower but perhaps more normal level. Further, the extent of exposure at low water may be subject to considerable variation depending on temporary changes in the contours of the foreshore.

12. Taxonomic.

Whereas some algologists, notably PAPENFUSS (1954) hold that the genus *Letterstedtia* should not be maintained but should be sunk in the genus *Ulva*, others have described additional species of *Letterstedtia* on supposed external resemblances only. J. AGARDH (1882 p. 175) described *L. petiolata* from the shores of „New Holland”, epiphytic on the stalks of a species of *Callymenia*; *L. japonica* from Japan was described by HOLMES (1895 p. 250), while more recently CHAPMAN (1956 p. 384) has recorded *L. petiolata* from New Zealand and has also described two new species from that country.

In AGARDH's description of *L. petiolata* the frond is said to be sessile on the top of the primary rootlike callus but the lower part „thickened above, gradually more expanded”. The leaves are described as arising „by evolution of marginal lobes” and as borne on petioles; the structure of the petioles and the primary stipe is described as different from that of the leaves, being thickened by „hyphoidal filaments densely crowded together”, the whole structure being analogous to the simpler and not very conspicuous stipes of other Ulvaceae, while the leaves are distromatic, the two cell layers separated by „an intermediate zone of hyaline intercellular substance generated by the walls of the cells having been thickened towards the inside.” His figures of longitudinal and transverse sections of the stipe are sketchy but apparently show a rhizoidal layer similar to but thicker than the corresponding structure of a robust *Ulva*. Dissection of the frond is said to arise by laciniation, rows of holes gradually elongating and coalescing until the frond is split. He is obviously misled by the description of the narrow strap-shaped basal fronds often found in *L. insignis* as stems. He points out that although ARESCHOUG obviously had the lower part of his plants he did not describe the internal structure. AGARDH's own specimen of *L. insignis* consisted only of the „upper part of the exceedingly elongated plant.” AGARDH concluded that his plant „scarcely belonged to the genus *Ulva*” and placed it tentatively in ARESCHOUG's genus although he clearly had grave doubts as to the correctness of this decision. DE TONI (1889 p. 139) quotes AGARDH's description *verbatim*, without comment.

WOMERSLEY (1956 p. 354), who has collected *L. petiolata* growing on the stems of *Cymodocea* in Port Phillip, follows PAPENFUSS and on somewhat insufficient grounds transfers it to *Ulva* as *U. petiolata*.

CHAPMAN (1956 p. 384) not only records *Letterstedtia petiolata* from New Zealand where it is apparently widespread, but describes two new species of *Letterstedtia* from that country, *L. stipitata* and *L. ulvoidea*.

It is difficult to understand CHAPMAN's statement (p. 383: – „*Letterstedtia* as originally described is typified by *L. petiolata*,” since „as originally described” by ARESCHOUG the genus was founded for the Natal plant *L. insignis*. CHAPMAN takes as the main characters which distinguish *Letterstedtia* from *Ulva* the presence of 1) a long narrow stipe and 2) „secondary interstitial cells” in the stipe region, neither of which features occurs in *L. insignis*, the type species of the genus. His photographs of *L. petiolata* show a pretty and very distinctive little plant which however bears remarkably little resemblance to *L. insignis* even in its external form; the same applies to his two other species. His figures of transverse sections of the fronds are sketchy but suggest the presence of an amorphous layer between the two cell layers while those of the stipe region, like AGARDH's, can be interpreted as indicating a very well developed rhizoidal central region (as indeed was described by AGARDH) and the cells which are variously described as „internal subsidiary cells”, „interstitial cells” or „the characteristic secondary cells figured by AGARDH” are merely various views of the rhizoids or the cells by which they are formed. With this interpretation WOMERSLEY agrees.

*Letterstedtia japonica*¹⁾ described by HOLMES (1895) from Japan is again a small plant (about 2 inches high according to HOLMES, 10 to 20 cm. according to OKAMURA) so far apparently obtained only from a restricted area on the Pacific shores of Japan. The frond is described by HOLMES as broadly flabellate, up to two inches long, deeply lacinate, olive green in colour. He goes on to describe the coalescing of numerous small slits in the thallus to cause deep splits and compares it with *Ulva rigida* but adds that its mode of growth is different. His drawings of transverse sections of the frond are not very helpful. OKAMURA (1928, 1936) amplifies HOLMES's description and figures a much larger plant. His figure of transverse sections of a young frond (Pl. 250 figs. 3 & 4) are interesting – figure 3 shows the marginal region of the frond with very thin walled cells; figure 4 of the same section but towards the centre of the frond shows thicker walled cells, the inner walls of which are apparently partly in contact, without any intervening hyaline layer. Unfortunately he does not give a figure of the structure in the thicker base of the flabellate frond. OKAMURA describes the fronds as solitary or few caespitose, with a small scutate disc and a short stem-like portion below. In the young plant the upper margin is entire and round but lacerated with age. The whole frond has scattered slits which enlarge and coalesce causing

¹⁾ I am deeply indebted to Dr. WM. RANDOLPH TAYLOR for sending me microfilms not only of the chief literature dealing with this species but also of his own Herbarium specimens, all, like the HOLMES specimen, from Enoshima.

the splitting of the frond. The colour is described as bluish green and the substance as coriaceous-membranous. As in the three other species attributed to *Letterstedtia* there are no varied types of lateral appendages; in all four apparently the frond is dissected by laciniation arising in a similar fashion, which may also occur occasionally in *L. insignis*, but whereas in the latter this plays but a very minor rôle in the formation of the complex thallus, in the other four species it is the main contributory factor leading to dissection of the thallus.

The photographs of W. R. TAYLOR's herbarium specimens show three similar plants, two considerably larger than that figured by OKAMURA, and give a very good idea of the form of the species.

It seems doubtful whether any of these species are correctly attributed to the genus *Letterstedtia*. Even the external morphology is very dissimilar and it seems probable that their identification as species of ARESCHOUG's genus will prove incorrect. The impression one gets is that in each case the author of the species felt that it was not an *Ulva* and simply placed it in *Letterstedtia* for lack of any other possible genus in which to put it. With the more exact knowledge of *L. insignis* now obtained a careful investigation of the structure alone of the four species would probably solve the question of their affinity and decide whether they should be included in one or other of the existing genera or placed in a new genus, or genera. Apparently nothing as yet is known as to reproduction in any of the four. From the external characters alone CHAPMAN's three plants might belong to a single genus, *L. japonica* possibly to the same but more probably to yet another genus.

Generic characters of *Letterstedtia*.

As a result of the present investigation additional characters for ARESCHOUG's genus *Letterstedtia*, distinguishing it from the genus *Ulva*, are regarded as: —

1. Greater complexity of external form with lateral appendages of several different types.
2. Massive attachment organ with wart-like secretions of callus entirely enveloping the primary holdfast and stipe of the sporeling.
3. Primary structure of the thallus distinguished by the inner walls of the two cell layers being in close juxtaposition, partially fused.
4. Differentiation into distinct meristematic and somatic (permanent) tissue, found throughout the plant, not only in the basal region.
5. Marginal regions of the whole plant meristematic giving rise to the lateral appendages mentioned in (1).
6. Growth in thickness found in any somatic part of the plant, brought about by a two-fold process: — a) the formation of rhizoi-

dal outgrowths from certain of the cells, accompanied by b) building up of a system of cross struts or trabeculae by deposition of wall-substance in the fused inner walls of other cells of the two cell layers.

7. Reproductive areas formed in any meristematic region; each fertile area sharply delimited by an outer and inner border of 1 to 4, most often 2, rows of cells; cells within the two borders simultaneously transformed into fertile cells.
8. After liberation of the swarmers, evacuated area sloughed off, the inner border forming the new margin of the frond; successive fertile areas often overlapping.

Other features found in *Letterstedtia insignis*, – the number of gametes in the gametangium (16, rarely 32), the characteristic frills always found on the straps and sometimes elsewhere in the thallus, the degree to which growth of the diffuse marginal meristem may result in appendages of various forms, may prove to be specific rather than generic characters.

The characters summarized above are in the opinion of the writer more than sufficient to distinguish *Letterstedtia* from *Ulva* and to justify the maintenance of ARESCHOUG's genus as distinct from the latter.

SUMMARY

1. *Letterstedtia insignis* is essentially a plant of the sub-littoral zone and its fringe; it has been recorded as growing at a number of stations on the east coast of the Cape Province, in quantity at Salt Vlei Bay and Waterloo Bay.
2. Periodic visits to these two localities have been paid during the period April 1958 to January 1959.
3. The alga exhibits a high degree of plasticity resulting in great complexity and variability in external form.
4. Plants in shallow water are typically bushy but may develop straps; fully adult plants may or may not have a bushy base. In deep water they attain considerable size (over 2½ feet) but have never been found reaching the size (4 feet or more) recorded by ARESCHOUG from Natal.
5. Except in the young sporeling the plant is thalloid, flattened throughout, with no distinct stipe.
6. The young parts of the plant show characteristic ulvacean structure but differ from *Ulva* in that even in the youngest parts the inner walls of the two cell layers are in contact with one another and partially fused.
7. Very early in development differentiation of meristematic and

somatic tissue begins to appear. Meristematic cells are thin walled each with a single parietal chloroplast lining the upper part of the cell and two or more pyrenoids, and are found in all marginal regions of fronds, leaflets and frills of the straps. Somatic cells have progressively thickened walls with many small granular chloroplasts and are found in the older parts of fronds and leaflets and the central region of the straps.

8. Growth in thickness is brought about in somatic regions by a two-fold process: – a) formation of rhizoidal outgrowths from the inner end of certain cells and b) deposition of wall substance in the fused inner walls of the two cell layers, resulting in the formation of an elaborate system of cross struts or trabeculae.
9. Growth in thickness may occur in any part of the plant even the extreme apical parts of the straps, usually becoming progressively more extensive towards the base of the frond. It is most apparent externally when it forms midribs in the straps.
10. A similar secretion of wall substance, both internally and externally, soon completely envelops the attachment organ and stipe of the young sporeling.
11. External secretion of the same substance builds up a complex wart-like structure so that the mature holdfast is large and conical in shape, the flattened fronds arising directly from its apex. The outer surface of the wart-like secretions are mucilaginous and become coated with sand particles which eventually impregnate the whole holdfast.
12. Wart like secretions often extend from the holdfast up on to the base of the frond strengthening it while others may form secondary attachment organs on the lower margins of the frond. Gall-like warts also occur on the upper parts of the thallus especially on the straps.
13. Reproduction may occur in any young meristematic part.
14. Reproductive areas are sharply delimited, small marginal arcs in leaflets and young parts of fronds, successive arcs often overlapping, marginal strips in the frills of straps. All the cells of the area except a couple of marginal rows form gametangia.
15. Recently divided cells become modified to form gametangia, the contents of each gametangium dividing into 16 (rarely 32) parts which round off, each containing a parietal chloroplast, a single pyrenoid and conspicuous eyespot.
16. When mature each develops 2 flagella, elongates and starts to move within the gametangium; activity is probably initiated by certain states of the tide.
17. After a short period of movement the 16 gametes escape through a raised pore in the centre of the outer wall.

18. Occasional fusion within the gametangium is regarded as a pathological phenomenon.
19. Liberation takes place more or less simultaneously from all the gametangia in a fertile area after which the colourless evacuated area with a narrow margin of green non-fertile cells is sloughed off.
20. On liberation „clumping” immediately starts, conjugation rapidly follows and the population of swimmers is soon composed mainly of quadriflagellate planozygotes.
21. Planozygotes settle down on any available surface, round off and secrete a wall.
22. This has been seen to take place on fertile fronds particularly on or near evacuated areas. Many of the rounded spores were small with a single eyespot, others larger with two eyespots. The eyespots were comparatively large and conspicuous and persisted for several days.
23. Both types of cells sometimes commenced to germinate, but most of the smaller ones soon degenerated.
24. It was concluded that the swimmers were either gametes or facultative gametes but whether they can continue to develop without conjugation is unknown. As a rule conjugation appears to be general.
25. No sign of the formation of quadriflagellate zoospores has yet been found.
26. Swimmers collected on slides and cover-slips settled down and were kept in culture in filtered sea-water plus a few drops of Schreiber's solution.
27. The spore enlarges, the contents usually becoming granular.
28. Elongation begins, a transverse wall cuts off the lower narrower part from the upper rounded cell; the former develops into an attachment organ, either a rhizoid or a polygonal cell, the latter by successive transverse divisions forms a filament of from 6 to 12 cells.
29. Longitudinal division starts a short way up the filament, extends downwards to the second cell and upwards, the last few cells remaining undivided. The end cell is dome-shaped and functions as an apical cell continuing to cut off segments parallel with its base.
30. From the daughter cells of the second cell rhizoidal processes sometimes grow down to help form the attachment organ.
31. Hyaline wall-substance is secreted round the foot of the basal cell gluing it to the glass.
32. Further divisions in the upper part of the filaments resulted in widening, the filament becoming 4 or 5 cells wide, while longi-

tudinal divisions extended into the monosiphonous tip and the apical cell itself finally divided longitudinally to form a meristem.

33. Flattening of the thallus was seen in sporelings grown *in situ* but has only once been obtained in culture; in some cultures various abnormalities appeared, some attributed to too free use of Schreiber's solution.
34. Naturally developing sporelings were found growing on *Placophora* epiphytic on *Codium Duthieae*; these provided a check on sporelings raised in culture.
35. Other species of *Letterstedtia* are compared with *L. insignis* and their probable affinities discussed.
36. The characters of *Letterstedtia insignis* are summarized, those considered to be generic enumerated and the conclusion reached that the retention of *Letterstedtia* as a genus distinct from *Ulva* is more than justified.

In conclusion I should like to thank Dr. WM. RANDOLPH TAYLOR once again for the microfilms of *Letterstedtia japonica*. To Dr. G. F. PAPENFUSS I owe gratitude for introducing me many years ago to the method used in the spore cultures and still more for the inspiration his keen interest in marine algae has always been. Finally I would like to thank those members of the staff and senior students of the Botany Department of Rhodes University who have helped in this investigation by sharing excursions to Salt Vlei and Waterloo Bays for the observation and collection of *Letterstedtia*.

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Thécamoebiens des terres de Belgique I

par

DIDIER CHARDEZ

THÉCAMOEBIENS DES TERRES DE BELGIQUE

Les premières recherches effectuées sur la Thécamoebologie du sol ont permis d'ouvrir de nouveaux horizons scientifiques, de préciser certaines idées relatives à l'évolution des formes et de démontrer les extraordinaires possibilités vitales de ces Rhizopodes encore si peu connus.

Les Thécamoebiens repris dans la première partie de cette étude proviennent de biotopes essentiellement terricoles, dans chaque cas je me suis assuré de l'absence de muscinées et de réserve d'eau en contact direct, ceci pour éviter les mélanges de faunes.

Il s'agit ici en quelquesorte de „prélèvements sondes” répartis dans les différents sols de diverses contrées de Belgique.

Il faudra des prélèvements plus nombreux, des études écologiques des terres plus poussées pour arriver à bien dégager les authentiques espèces terricoles des espèces provenant d'autres milieux.

La faune Thécamoebienne des terres est bien caractéristique et très particulière. Les récentes études de L. BONNET & R. THOMAS (1) ont démontrés outre l'importance des études du sol la réalité de la présence dans ce milieu d'espèces typiques morphologiquement conçues pour vivre dans ce milieu ou la natation est pratiquement impossible. Dès lors bien des problèmes se posent sur la biologie générale d'une telle faune qui s'avère aussi importante que les faunes muscicoles ou aquatiques.

Certains caractères morphologiques tendent à prouver un mode de vie fixé, par exemple les tendances à la cryptostomie et à la plagiostomie, ainsi que le font remarquer L. BONNET & R. THOMAS.

Certaines espèces sont incontestablement terricoles toujours présentes en populations dans les terres, d'autres plus rares semblent là par erreur ou faute de mieux mais proviennent presque toujours de milieux aériens.

Il est à remarquer que chez beaucoup d'espèces que l'on peut considérer comme essentiellement terrioles le pseudostome est réduit à sa plus simple expression, simple fente, petit trou le plus souvent encore cloisonné intérieurement en véritable labyrinthe ne permettant pas ou très difficilement l'émission de pseudopodes.

TECHNIQUE

L'examen microscopique des terres par les procédés classiques ne donne rien et ne peut en aucun cas mettre en évidence la faune particulière et souvent fort riche des sols, il est donc nécessaire d'avoir recours à une technique particulière et nouvelle de préparation.

Mon procédé du microséparateur à air ou à gaz est basé sur la double propriété qu'ont les Thèques de pouvoir flotter à la surface d'un liquide et de répondre parfaitement aux lois des tensions superficielles. L'échantillon de terre préalablement desséché est agité énergiquement dans trois fois son volume d'eau distillée environ, puis, introduit par un tamis à grosses mailles (passoire à thé) dans un tube qui sera obturé par un bouchon percé de trois trous, par le premier trou descend jusqu'au fond du tube un tuyau d'arrivage d'air, du second trou sort un fin tube partant du niveau de la base du bouchon et qui peut être obturé par le doigt ou fermé par un robinet, dans le troisième trou passe un tuyau qui partant du fond du tube est recourbé en U et aboutit à une large éprouvette.

Il suffit le tout mis en place de laisser barboter l'air fournit par une pompe d'aquarium pendant une ou deux minutes, on obture ou on ferme le robinet: du second tube, l'air s'accumulant alors dans le tube principal pousse le tout par le tube en U dans l'éprouvette.

Les déchets les plus lourds restent dans le tube principal, tandis que les Thèques flottant dans l'eau distillée se réunissent par les lois des tensions superficielles au niveau de capillarité dans l'éprouvette, ou elles seront prélevées en masse à la pipette.

L'air peut-être remplacé par un gaz, Hydrogène ou Oxygène par exemple, il suffit de remplacer la pompe à air par un appareil générateur d'un de ces gaz.

LES PRELEVEMENTS

A, Sols humifères

- | | |
|--|-------------------|
| 1. Terre 10cm de prof en forêt (Ardenne) | pH 4 ⁵ |
| 2. Terre de surface en forêt (Jalhay) | pH 4 ⁵ |
| 3. Terre de surface en forêt (Fagnes) | pH 6 |
| 4. Terre 15 cm de prof en sapinière (Fagnes) | pH 6 |

5. Terre de surface en sapinière (Oneux)	pH 6
6. Terre cultivée de jardin (Mangombroux)	pH 6 ⁵
7. Terreau naturel dans une haie (Heusy)	pH 5
8. Terreau compost de serre	pH 5
9. Terre 20 cm de prof. sous végétation (Jalhay)	pH 5 ⁵
B. Sols mixtes	
10. Terre argileuse sous gazon (Heusy)	pH 6 ⁵
11. Terre argileuse chemin (Heusy)	pH 6
12. Terre schisteuse (Verviers)	pH 6
C. Sols vierges	
13. Terre eolienne sur rocher calcaire (Heusy)	pH 7 ⁵
14. Terreau sur rocher calcaire (Stembert)	pH 6
15. Terre eolienne dans une souche d'arbre mort (Fagne)	pH 6 ⁵
16. Terre eolienne au creux d'un arbre (Heusy)	pH 6
D. Sols squelettique	
17. Terre de désagrégation de roche (Mangombroux)	pH 5
18. Terre de désagrégation d'un vieux mure (Tongres)	pH 5
E. Sols sableux	
19. Terre sableuse en surface (Percrot)	pH 6
20. Terre sableuse en surface forêt (Pecrot)	pH 5
21. Terre de surface en sapinière (Bourg-Leopold)	pH 5 ⁵
22. Sable d'origine souterraine (Mangombroux)	pH 6
23. Sable de surface (Bourg-Leopold)	pH 6 ⁵

LISTE DES ESPECES

Les n° se rapportent aux prélèvements

Arcella hemisphaerica PERTY I.

Types dont les dimensions moyennes sont légèrement inférieures à la moyenne, contour du pseudostome assez irrégulier teinte assez foncée.

Assulina muscorum GREEFF. 3, 4, 14.

Centropyxis aerophila DEFLANDRE 1, 2, 3, 4, 5, 6, 13, 14, 16, 17, 18, 20, 21.

Très fréquente en général dans les terres.

Centropyxis aerophila var. *globulosa* BONNET & THOMAS 20.

Centropyxis aerophila var. *sphagnicola* DEFLANDRE 3, 4, 5, 9.

Centropyxis platystoma (PEN.) DEFLANDRE 20.

Centropyxis plagiostoma BONNET & THOMAS 20 Fig. 3 diam. 100 microns

Centropyxis plagiostoma var. *terricola* BONNET & THOMAS 8, 15, 16. Fig. 4.

Très caractéristique et plus fréquente de l'espèce type diam. 60 microns.

Centropyxis ecornis var. *Leidyi* THOMAS 14, 17.

Corythion dubium TARANEK 2, 4, 10.

Corythion dubium var. *aerophila* DECLOITRE 3.

Diffugia oblonga EHRBG. 1.

Diffugia oblonga var. *bryphila* PENARD 4.

Ces deux espèces ont été vues uniquement dans un seul prélèvement mais en quantité suffisante pour être signalées; toutefois je ne tire aucune conclusion n'ayant vu que des coquilles vides.

Diffugiella oviformis var. *fusca* PENARD 5.

Euglypha compressa CARTER 7.

Euglypha ciliata (EHRBG.) PERTY 5.

Euglypha rotunda WAILES 1, 2, 3, 8, 9, 20.

Espèce très fréquentes dans les terres, beaucoup de petites formes.

Euglypha strigosa (EHRBG.) LEIDY 2, 4.

Euglypha strigosa fa. *glabra* WAILES 3, 14.

Geopyxella sylvicola BONNET & THOMAS 1, 2, 11. Fig. 6.

Thèque d'aspect lisse souvent foncée le pseudostome est peu invaginé circulaire, j'ai remarqué sur plusieurs exemplaires un épaississement chitineux du contour du pseudostome.

Heleopera petricola LEIDY 9.

Nebela militaris PENARD 3, 4.

Nebela parvula CASH 2.

Nebela tinctoria (LEIDY) AWERINTZEW 3, 4, 5, 9, 14.

Nebela tinctoria fa. *stenostoma* JUNG 3, 4, 14.

Plagiopyxis callida PENARD 5, 7, 8, 9, 10, 11, 13, 14, 17, 20.

Plagiopyxis callida var. *grandis* THOMAS 2, 3, 5, 7, 10, 13, 14.

Plagiopyxis declivis THOMAS 1, 2, 3, 4, 5, 7, 8, 9, 14, 15, 16.

Plagiopyxis declivis var. *oblonga* THOMAS 7 fig. 2.

Plagiopyxis penardi THOMAS 14.

Les genres *Plagiopyxis* et *Phryganella* sont, je crois, les plus représentatifs de la faune terricole, observées dans presque tous les prélèvements en très grandes populations.

Phryganella acropodia HERTW. LESS 2, 3, 5, 6, 7, 9, 13, 14, 15, 16, 19, 20, 21.

Tracheleuglypha acolla BONNET & THOMAS 3, 8, 16, 20.

Trinema enchelys (EHRBG.) LEIDY 5, 7, 8, 14, 16.

Trinema enchelys var. *biconvexa* AWERINTZ. 5, 9.

Trinema complanatum PENARD 1, 3, 4, 5, 9, 14, 16, 20.

Très fréquente souvent en grande population.

Trinema complanatum var. *globulosa* var. nov. 1, 14, 16.

Cette nouvelle variété de *T. complanatum* Fig. I se distingue facilement par son allure plus courte et plus globuleuse, le pseudostome garde les proportions du type, mais le dos de la thèque semble plus bombé, le revêtement est fait d'écailles petites, circulaires, irrégulières de taille et mélangées à d'autres écailles ovales.

Mensurations:	L 30	H 20
	L 35	H 20
	L 37	H 24

Trigonopyxis arcula (LEIDY) PENARD 2, 3, 4, 5, 9, 14, 15, 16.

Observé en population très nombreuse, je pense intéressant de signaler quelques grands individus de 170 à 190 μ dont la thèque de teinte très foncée est formée de grandes plaques amorphes et épaisses donnant à ces grands organismes un aspect plus robuste que pour les *T. arcula* de tailles normales.

Ces grandes formes n'ont pas été vues en population mais toujours isolées parmi les formes normales c'est pourquoi je ne crois pas utile de voir là une nouvelle variété.

La Fig. 5. représente quelques formes de pseudostome de *T. arcula* observés.

ESSAI SUR UNE ECOLOGIE DU SOL

Seul un ensemble bien plus considérable de récoltes permettra d'établir une base solide à l'écologie des Thécamoebiens terricoles, toutefois en éliminant les formes trop ubiquistes pour servir de départ à une faune terricole il me reste quelques espèces intéressantes, qui ajoutées à la liste publiée par L. BONNET & R. THOMAS (1955) porte celle-ci de 14 à 26 représentants de la faune terricole (les espèces marquées* ont été vues uniquement par L. BONNET et R. THOMAS).

**Arcella arenaria* 17 % des récoltes B. T.

Arcella hemisphaerica 4 % des récoltes seulement mais intéressante par sa population.

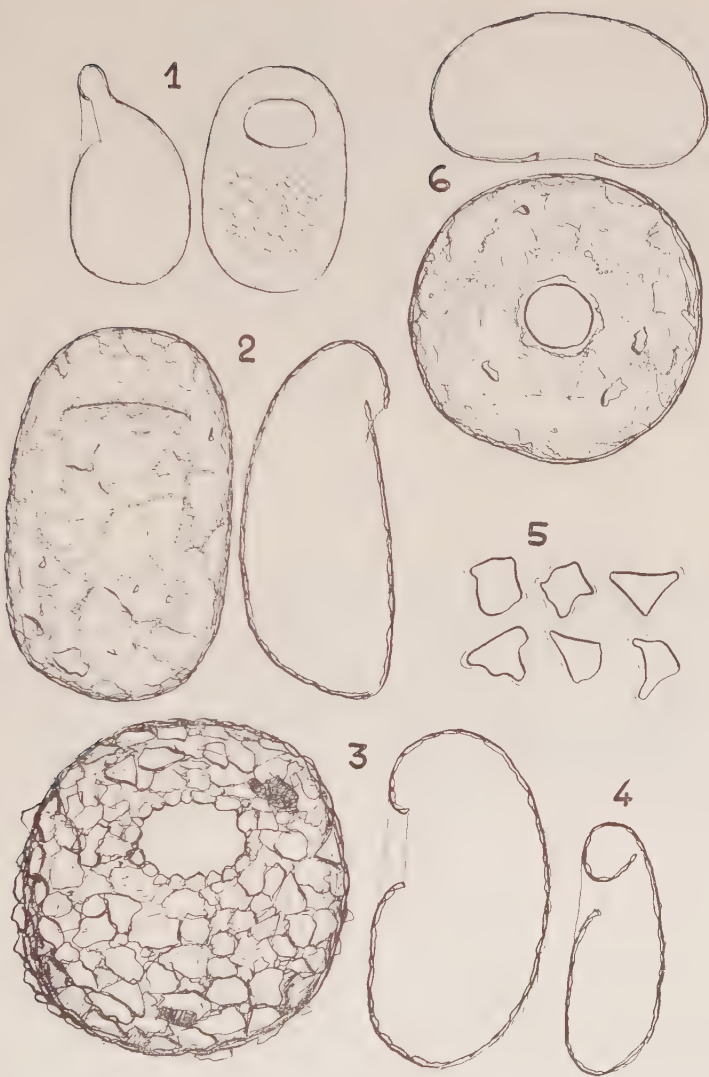
**Bullinularia indica* 12 % des récoltes B. T.

Centropyxis aerophila et var. *globulosa* et *sphagnicola* 66 % des récoltes.

Centropyxis plagiostoma 4 % et 10 % des récoltes B. T.

Centropyxis plagiostoma var. *terricola* 12 % des récoltes.

REMARQUE. Les prélèvements 12, 22, 23, ne contenaient aucun Thecamoebien.



**Heleopera petricola* var. *humicola* 13 % des récoltes B. T.
 **Hyalosphenia insecta* 2 % des récoltes B. T.
 **Hyalosphenia subflava* 10 % des récoltes B. T.
Plagiopyxis declivis et var. *oblonga* 44 % des récoltes.
Plagiopyxis callida et var. *grandis* 60 % des récoltes.
Phryganella acropodia 50 % des récoltes.
Trigonopyxis arcula 28 % des récoltes.
Trinema lineare 60 % des récoltes.
Trinema complanatum et var. *globulosa* 32 % des récoltes.
Trinema enchelys et var. *biconvexa* 4 % des récoltes.
Euglypha rotundata 24 % des récoltes.
Tracheleuglypha acolla 16 % des récoltes.
Nebela tincta et fa. *stenostoma* 28 % des récoltes.

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The development of eggs of *Chirocephalus diaphanus* Prévost in relation to depth of water

By

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INTRODUCTION

In a previous paper (HALL, 1959) the author described the phenomenon of delayed development in the eggs of *Chirocephalus diaphanus* and suggested that there was a correlation between this and the depth of water overlying the eggs. A distinction was drawn between this phenomenon and that of delayed hatching described earlier by the author (HALL, 1953). The present paper describes some further observations on the relationship between delayed development and the depth of water overlying the eggs.

MATERIAL AND METHOD

Eggs were obtained from a pair of *Chirocephalus* brought in from an outdoor aquarium and placed in a beaker of water in the laboratory. Between two and three hundred eggs were laid in the ensuing twenty-four hours. Five glass tubes of approximately 2.5 cm. in diameter were filled with water from the aquarium to depths of 5, 10, 15, 20 and 25 cms. and about fifty eggs were placed at the bottom of each tube. It was noted that the surface sculpturing of the eggs was somewhat obscured by green algal and faecal material, i.e. the eggs were 'dirty' in the sense used by the author (HALL, 1959).

The tubes were examined daily, and any nauplii which had hatched were removed. After a period of fourteen days had elapsed ten eggs from each tube were removed and placed in a watchglass filled with water from the same aquarium. The watchglasses were kept in the

laboratory under daily observation and the dates on which the break appeared in the outer covering of the egg were noted. Further groups of ten eggs were removed from the tubes at fortnightly intervals and treated in the same way. The mean laboratory temperature throughout this period was 22°C.

RESULTS

By the end of the first period of fourteen days no hatching had occurred and none of the eggs in any of the tubes showed any signs of the break in the outer covering. Table I shows the time taken for the break to appear in the various groups of eggs removed from the tubes after fourteen days. It will be noticed that the breaking stage was reached in a very short time (1.7 days) in the eggs which had been kept at a depth of 5 cm., while those kept at a depth of 10 cm. required on the average a little over six days. The period required by eggs from depths of 15, 20 and 25 cms. was a little greater, the average being about eight days, with no significant difference between the periods required by the three groups.

TABLE I

Showing time required by eggs to reach breaking stage in shallow water after 14 days immersion in various depths of water. The table shows the total numbers of days required for development at both depths and the number of days by which this exceeds the normal.

Depth of water overlying eggs.	5 cm.	10 cm.	15 cm.	20 cm.	25 cm.
Number of days required for development in shallow water.	1.7	6.3	8.0	8.1	8.3
Total number of days required including 14 days at greater depths.	15.7	20.3	22.0	22.1	22.3
Number of days delay beyond normal period of eight days in shallow water.	7.7	12.3	14.0	14.1	14.3

On the first day of the second period of fourteen days numerous nauplii were seen swimming in the tube containing a 5 cm. depth of water, but none were seen in any of the other tubes. By the third day

35 of the 40 eggs had hatched. This period compares closely with the mean period of 1.7 days required by those transferred to shallow water.

On the seventh day three nauplii were seen in the tube containing a 10 cm. depth of water, and by the eighth day six more had hatched and most of the remainder had reached the breaking stage. Again this period is closely comparable to the time required by the eggs transferred from this depth to shallow water after fourteen days.

Thus, since both these groups of eggs had already undergone a period of fourteen days immersion at depths of 5 cm. and 10 cm. respectively it appears that development at a depth of 5 cm. took about sixteen days to reach the breaking stage, while at a depth of 10 cm. the corresponding period was about twenty days.

TABLE II

Showing the number of days required by eggs to reach breaking stage in shallow water after immersion for various periods in different depths of water.

	Depth of immersion.			
		15 cm.	20 cm.	25 cm.
Number of days immersed	28	3.1	7.3	7.0
	42	2.4	7.7	8.2
	56	1.9	7.7	7.6

Table II shows the time required to reach the breaking stage in shallow water of successive batches removed from depths of 15, 20 and 25 cms. after periods of 28, 42 and 56 days immersion respectively. It will be noticed that in the case of the eggs kept at a depth of 15 cm. the time required in shallow water to complete development is reduced as the initial period of immersion at the greater depth is increased. On the other hand there are no significant differences discernable in the times required for completion of development in shallow water with different periods of immersion in deeper water in the case of eggs kept at 20 and 25 cms. respectively. As was pointed out above, all the eggs initially kept at a depth of 5 cm. and 10 cm. had completed their development before the period of twenty-eight days had elapsed.

DISCUSSION

The author (HALL, 1959) found that eggs kept in watchglasses at a laboratory temperature of 22°C. developed to the breaking point in about eight days. In the experiments described above, carried out at the same temperature, no eggs had reached the breaking stage at the end of fourteen days: this stage was reached after about sixteen days by the eggs immersed to a depth of 5 cm. It would appear therefore that immersion at this depth increases the time required for development by approximately eight days. Since breaking of the outer covering, and hatching, occurred both in the eggs later transferred to shallow water and in those retained at the depth of 5 cm., and the time required for development was the same, slow development must have continued during the whole period for which the eggs were immersed.

Further reference to Table I shows that the corresponding delay for a depth of 10 cm. was about 12 days, while those for 15, 20 and 25 cms. were effectively the same at about fourteen days. This suggests that the retarding effect of depth begins to show at depths of between one and five cms., and that it increases up to a depth of about 15 cms., but that beyond this point there is little increase in the effect.

A consideration of the data shown in Table II, which gives the lengths of time taken in shallow water for development of eggs kept for various periods at greater depths appears to support this. Thus, the longer eggs were kept at a depth of 15 cms., the shorter the period they subsequently required for completion of development. This suggests that even at a depth of 15 cm. some slow development occurs. In view of the fact that eggs kept at depths of 20 cm. and 25 cm. for various periods showed no significant differences in the times required for development, it would appear that at these depths there is almost complete cessation of development.

These two sets of results in general confirm the retarding effect associated with depth of water on rate of development of the eggs, and they clearly suggest that the maximum effect is produced by a depth of 20 cm. or more, and that even a depth of 5 cm. has some, though a rather smaller, retarding effect.

Observations by workers in America on two species of branchiopod crustacea support the view that there is a relationship between the depth of water and the development of the eggs. CASTLE (1938), working with *Eubbranchipus vernalis* (VERRIL), found some evidence that eggs had hatched in an aquarium in which the depth of water had been greatly reduced, though no details of the depth are given. AVERY (1939) refers to the keeping of eggs of the same species in finger bowls until they were needed, though he does not quote the

depth of water. He also found that eggs transferred to a watchglass of water hatched. WEAVER (1943), also working with this species, found that eggs collected from aquaria and kept in plain water remained for as much as nine months without hatching. However, no details are given of the depths at which they were immersed. MOORE (1951) found that eggs of *Streptocephalus seali* which had been immersed for several weeks hatched after the volume of water covering them had been halved by evaporation.

SUMMARY AND CONCLUSION

1. The effect of keeping eggs of *Chirocephalus diaphanus* in different depths of water for various periods of time is investigated.
2. It is found that at depths of 5, 10 and 15 cms. the period required for development and hatching is greater than that required in shallow water, and that the time required increases with increase of depth over this range.
3. At depths of 20 cm. and greater little development appears to occur: eggs transferred from these depths to shallow water require the normal time for development.
4. Reference is made to several other examples in the literature which appear to indicate that a similar phenomenon may occur in the development of eggs of other Branchiopods.

RÉSUMÉ

1. L'effet de maintenir les oeufs de *Chirocephalus diaphanus* dans plusieurs profondeurs différentes de l'eau pour les périodes de temps variées est examiné.
2. Dans les profondeurs de 5, 10 et 15 cms. la période demandée pour le développement et pour l'éclosion est plus longue que celle dans l'eau profonde, et le temps demandé augmente avec l'augmentation de la profondeur d'un bout à l'autre de cette étendue.
3. A la profondeur de 20 cms. et plus il-y-a manifestement un développement: les oeufs transférés de ces profondeurs dans moindre l'eau peu profonde se développent dans la période normale.
4. On cite plusieurs autres exemples qui manifestement indiquent qu'il peut arriver un phénomène semblable dans les développement des oeufs des autres Branchiopoda.

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The life-history of *Ameletus inopinatus* (Siphonuridae, Ephemeroptera)

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INTRODUCTION

Ameletus inopinatus EATON, 1887 is the only arctic-alpine species of the order Ephemeroptera recorded from the British Isles. It occurs in the Vosges mountains (EATON 1883—88, SCHOENEMUND 1930a); in the mountains of the Black Forest (EATON 1883—88, SCHOENEMUND 1930a, EIDEL 1933); in Czechoslovakia in the High Tatra to the east (SCHOENEMUND 1930a), in Moravia in the centre (ZELINKA 1951) and in the Böhmerwald mountains to the west (WINKLER 1956). SCHOENEMUND (1930b) and TIENSUU (1937) record it from Norway. In Britain it is usually found in streams at high altitudes (BROWN 1937, HARRIS 1952, KIMMINS 1954 and MACAN 1959) but has been recorded from several lochs not far above sea level in the extreme north-west of Scotland (MACAN 1959). In the Lake District it has not been found in streams below 1000 ft (300 m) (MACAN 1951).

THE STREAM

Whelpside Ghyll rises at about 2,820 ft (859 m) on the western slopes of Helvellyn in the English Lake District. At 2,170 ft (661 m) it is joined by a tributary which has its source at about 2,350 ft (716 m). Just below the confluence the stream falls steeply, with a gradient of 1 in 2.9, for about 1,000 ft (304 m), afterwards resuming a less precipitous course, 1 in 4, into Thirlmere at 600 ft (182 m) near the village of Wythburn. The observations were carried out in the tributary above the confluence, where it consists mainly of small pools and stretches of shallow, fast-flowing water connected by small waterfalls. The average gradient in this stretch, from its source to

just below the confluence, is 1 in 4.7. It is about 1 m wide with depths varying from a few to about 50 cm in the deeper pools. The bed varies from gravel to quite large stones, a few big enough to require two hands to lift them. *Fontinalis* sp. was present on the larger stones. Two stations were chosen, St. II being about 45 m upstream from St. I.

METHOD

Collections were made with a net having 20 threads to the inch and in 1958 a fine net with 180 threads to the inch was also used. These are the two standard pond nets produced by the Freshwater Biological Association. Dr MACAN has asked me to point out that he was using similar nets and his figure of 160 is an error (MACAN 1957). I used the collecting technique described by MACAN (1958), turning stones as I moved upstream and catching the animals thus disturbed. I also swept under the banks where undercutting had occurred and, in the deeper pools, held the net against the bottom and disturbed the area in front. Collecting lasted for five minutes at each station and the collections were sorted in the laboratory. The specimens of *A. inopinatus* were measured, usually with the naked eye, from the front of the head to the base of the caudal filaments. Collections were made during 1957, 1958 and early 1959, generally once a month except in the winter. In April 1958 a „pyramid” emergence trap (MUNDIE 1956, fig. 3) was placed in the lower pool of St. II and in May another trap was put in position about 10 m upstream. The second trap was square in plan and had a catching area of 1472 sq. cm. It was of the floating box-trap type (MUNDIE, 1956 p. 9) being supported by corks placed round the perimeter. The sides were of Tygan Screencloth, a plastic fine-meshed gauze and the roof was of transparent celluloid.

THE COLLECTIONS

Fig. 1 shows the numbers of *Ameletus inopinatus* caught each collection at St. I arranged in mm size groups and also the numbers of adults caught by the traps during the emergence period. Because the traps were not in use during 1957 and, as the course of events during that year was similar to that of 1958, detailed information about the 1957 collections has been omitted. October 1957 was chosen as the starting point because that was the month when small nymphs first appeared. As can be seen from the figure, the species has a rather long emergence period, from late May to late August, growth starts

in the winter and the cycle is completed in a year. Events are not, however, quite as straightforward as they at first seem. Presumably the first specimens to hatch in the autumn of 1957 had been missed, the collections in September having been made with a coarse net. Fine-net collections in August and September 1958 showed this

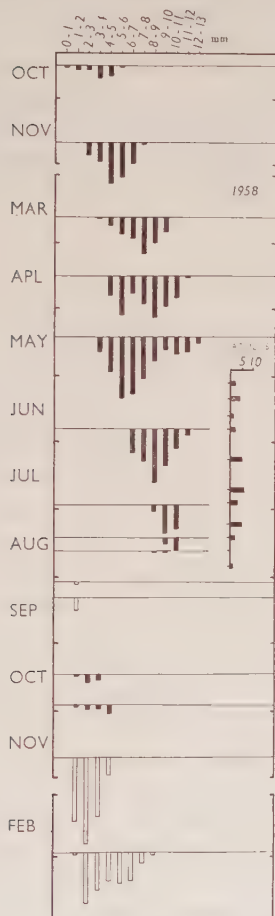


Fig. 1.

The numbers of *Ameletus inopinatus* caught each collection at St. I arranged in mm size groups and the numbers of adults caught by the traps during the emergence period. The unshaded histograms refer to collections made with a fine net.

assumption to be correct, the first small ones being found on 2 September. Growth continued throughout the winter of 1957—58 but in April another peak appeared besides the one at 8—9 mm and in the May collection this lower peak was more accentuated. In February the following year the fine net collection shows a similar pattern and gives every indication that two peaks will develop as in the April and May collections of 1958. What causes the two peaks? One possibility is that only some of the nymphs have been growing as happens with the damselfly *Pyrrosoma nymphula* (MACAN,

unpublished records). Another is that there is a peak of hatching in the autumn and another early the next year, with little or no hatching in mid-winter.

TABLE I

Body lengths in mm of adult *Ameletus inopinatus* (from front of head to base of caudal filaments).
i = imago. s = subimago.

	7.0	7.5	8.0	8.5	9.0	9.5	10.0	10.5	11.0	11.5 mm
10 June							♂ i ♀ i		♂ s	♀ s
18 June								♀ s		
24 June							♂ s		♂ s	
8 July			♀ s			♀ s				
29 July		♀ s	♂ s		♂ s					
7 August	♂ s		♂ s		♂ s ♀ s		♂ s			
13 August			♂ s ♂ s							
26 August		♀ s								

A study of the adults supports the second possibility. Table I shows that the early ones are large and the later smaller. If the larger adults are from nymphs that have grown throughout the winter, and the smaller ones from eggs that hatched later, there must have been bursts of hatching at two different times. In May the largest nymphs were 12—13 mm long, emergence could be expected to commence but no adults were taken in the traps until 3 June although cast skins and a male subimago were found on the surface of the lower pool of St. I on 27 May. Emergence continued until late August although numbers caught at any one time by both traps never exceeded 6 specimens. The emergence period then is short compared with the egg-hatching period. EIDEL (1933) gives July and August as the emergence period and June and early July are given by HARRIS (1952) and KIMMINS (1954). BROWN (1937) has taken subimagines as early as 15 May.

Mating and oviposition were not observed by the writer and the only adult seen, apart from those trapped, was the subimago of 27 May. BROWN (1937) mentions this elusiveness of the adults.

SUMMARY

Ameletus inopinatus has an emergence period lasting from late May until late August. That the hatching of eggs goes on from September to February is deduced from the capture of tiny nymphs in these months. Judging from the numbers in the different size groups, there is a peak of egg hatching in the autumn and another early the following year with a period in between with little or none. Nymphs growing throughout the winter produce the larger adults in May and early June, and it is thought that all the smaller adults emerging later originated from eggs that hatched later. The life cycle is completed in a year.

ZUSAMMENFASSUNG

Die Flugperiode von *Ameletus inopinatus* dauert von Ende Mai bis Ende August. Dass das Schlüpfen aus dem Ei von September bis Februar stattfindet, wird aus dem Fang von winzigen Nymphen während dieser Monate geschlossen. Die Beurteilung der Zahlen der verschiedenen Grossengruppen zeigt ein Maximum des Schlüpfens aus dem Ei im Herbst und ein zweites früh im folgenden Jahr. In der Periode zwischen diesen beiden Maxima schlüpfen wenig oder keine Tiere. Nymphen, die während des ganzen Winters wachsen, liefern die grösseren Imagines im Mai und Anfang Juni. Es wird angenommen, dass alle kleineren Imagines, die später schlüpfen, aus Eiern stammen, die später geschlüpft sind. Der Lebenszyklus in einem Jahr vollendet.

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Zeitschrift für Kryptogamenkunde, herausgegeben von F. MATTICK und J. GERLOFF; Verlag von H. R. Engelmann (J. Cramer) Weinheim/Bergstrasse.

The new review dedicated to cryptogamic botany is in reality a continuation of the old so well known „Hedwigia”. Of „Hedwigia” 81 complete volumes appeared, as well as issues 1 and 2 of vol. 82. „Nova Hedwigia” will carry on the tradition of the disappeared review and we can only hope that it will be for cryptogamic botany of as great value as its predecessor.

The first issue (which costs 20 Mark) gives articles of: SKUJA, ETTL, GERLOFF, HORTOBAGYI, FOTT a.o.

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P. v. O.

I. SPARING: Die Larven der Hydrachnellae, ihre parasitische Entwicklung und ihre Systematik. Mit 103 Abbildungen im Text. III, 168 S. gr. 8°, 1959. Broschiert 13.85 DM.

Parasitologische Schriftenreihe, Herausgegeben von Prof. dr. W. EICHLER, Kleinmachnow - Prof. dr. E. W. SPREHN, Celle - Prof. dr. H. J. STAMMER, Erlangen.

In this study the author gives the first essay of a system of the larves of Hydrachnellae. It is a very important work and all specialists must have it. But it also contains many new conceptions of which only experience will show if the views of the author can be accepted. Beside the systematical conception, there are also results of research on ecology and oviposition, the periodicity of oviposition and other questions. It is quite probable that this book will be the starting point of a series of studies on the larves of hydrachnellae, so that everyone who takes interest in this field will find here a lot of ideas and suggestions.

P. v. O.

Prof. Dr. O. HARNISCH: „Rhizopoda” „Die Tierwelt Mitteleuropas” heraus-
geben von P. BROHMER, P. EHRLICH, G. ULMER
1. Band Lief. 1b. Urtiere – Hohltiere – Würmer Verslag Quelle & Meyer,
Leipzig 12 DM. 75p. 26 tables.

The collective work „Die Tierwelt Mitteleuropas” is being continued after a long interruption caused by the war. One of the new issues that interests the readers of HYDROBIOLOGIA has just left the press. It is that by Prof. HARNISCH – who is working at Plön – in which the Rhizopods are treated.

Ever since the appearing of the book of HOOGENRAAD & DE GROOT completed in 1938, no new treatise on Rhizopods was written. Probably there will appear one within short, but for the moment being, the work by Prof. HARNISCH is most up to date.

As was to be expected, it has a personal character. The species are followed by a list of varieties that are very briefly characterized.

For some details the author seems to have consulted an old nomenclature, e.g. „*Quadrula*” now is being called „*Quadrulella*”. In spite of small remarks to be made, Prof. HARNISCH’s handbook is quite good; it contains the best dichotomical tables available. Rhizopoda amoebina are very well described. The author gives also dichotomical tables for Heliozoa.

P.v.O.

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